Studies in Wheat Stem Rust
(Puccinia Graminis Tritici)

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PART I

BIOLOGIC FORMS OF WHEAT STEM RUST (PUCCINIA GRAMINIS TRITICI) IN WESTERN CANADA

Introduction

The control of wheat stem rust, caused by *Puccinia graminis tritici* Erikss, and Henn., is one of the major problems in Canadian agriculture. The problem in Canada is somewhat different from that in the United States because there are not sufficient barberry bushes to account for the great rust epidemics which sweep across the country from time to time. We have, moreover, no evidence to show that urediniospores, after pasing through the long, cold Canadian winter, with the alternate thawing and freezing in the spring, can cause successful infection on the young wheat plants. The generally accepted hypothesis is that the rust moves northward from the United States. If this is true, then the breeding of resistant wheats is the only practical way of solving the problem. That resistant varieties of wheat can be secured has been conclusively shown by Biffen, in England, who succeeded in developing a variety which was not only highly resistant to *Puccinia glumarum* Schm., but which was also commercially desirable. It would seem, then, that the problem is primarily one for the plant breeder. Possible complications and difficulties in the breeding of resistant varieties were indicated by the common experience that a variety resistant in one locality may be susceptible in another. The explanation of this seeming anomaly was made evident by the discovery by Stakman and his co-workers (56 et seq.) that there are several biologic forms of *Puccinia graminis tritici*, and that a variety resistant to one form may be quite susceptible to another.

It is, therefore, quite evident that the plant pathologist must do pioneer work in the analysis of biologic strains of rust before the breeder can be assured of effective results in producing resistant varieties of wheat.

The investigation here reported attempts such an analysis. It was begun at Macdonald College in 1918, and continued at the University of Saskatchewan and the University of Minnesota, until the present time (April, 1922).

Acknowledgments

The work was carried on under the auspices of the Honorary Advisory Council for Scientific and Industrial Research of Canada. Collections were facilitated by an appointment from the Canadian Department of Agriculture in the summer of 1920. The writer wishes to acknowledge the kind help received from Dr. E. C. Stakman and Mr. M. N. Levine, Cereal Investigations, Bureau of Plant Industry, at the University of Minnesota, and Professor W. P. Fraser, Dominion Laboratory of Plant Pathology, at the University of Saskatchewan.

Historical Summary

The phenomenon of biologic specialization in rusts has for many years attracted the attention of workers in pure and applied biology because of the light which it throws on the parasitic behaviour of fungi, and lately because of the practical application which may be made by the establishment of a basis for rust resistance.

Eriksson (15) was the first to show definitely that biologic specialization occurs in cereal rusts. He worked with *Puccinia graminis* Pers. and in 1894 showed that what was usually considered as one species attacking all the common cereals in reality consisted of several pathological strains or biologic races.

This discovery stimulated much research, and various biologists in Europe and the United States began work in earnest upon this problem. The whole field of biologic specialization has been carefully reviewed by Reed (49), and specialization in the cereal rusts by Stakman (52) and a detailed review, therefore, is not given here. Only those papers are reviewed which are directly relevant to the present problem.

Until 1916 the existence of biologic forms of *P. graminis* on wheat was not suspected. During that year a form of stem rust was collected by Stakman and Piemeisel (58) in the Palouse district of Washington and Idaho to which certain varieties of wheat were almost immune,

although these same varieties were readily susceptible to a form of rust collected at St. Paul, Minnesota. This susceptibility and immunity of the same variety of wheat in different localities was most readily explained by assuming the existence of more than one biologic form of the rust fungus, each form capable of affecting only certain wheats.

Since 1916, by far the most extensive work on biologic specialization of *P. graminis* has been done by Stakman and his co-workers (36, 37, 54, 55, 56, 57, 58, 59, 60) at the University of Minnesota, in cooperation with the United States Department of Agriculture. These investigators demonstrated conclusively the existence of about a dozen biologic forms, which differ from one another chiefly in their infection reactions towards wheat varieties. Experiments were conducted with about twenty-five strains and varieties of wheat, including representatives of *Triticum aestivum*, *T. durum*, *T. compactum*, and *T. monococcum*. All degrees of resistance and susceptibility to the biologic forms then known were met with, from complete resistance in Khapli (C.I. 4013) an emmer, to complete susceptibility in the club wheats. The remainder of the varieties fell into an intermediate group, susceptible in varying degrees to some forms of the rust, and resistant or immune to others.

To test the question of the possible temporary character or mutability of these forms, Leach (36) and Stakman and Levine (60) studied the behaviour of one of them, *Puccinia graminis tritici compacti*, and found it to be as constant parasitically as the forms originally described by Eriksson (15).

Recently Mains and Jackson (39) have shown that *Puccinia triticina* Erik. consists of at least two biologic forms.

In 1919, Hoerner (31) found evidence of the existence within oat varieties of at least four specialized races in *Puccinia coronata* Cda., which next to *Puccinia graminis* is probably the most extensively investigated rust from the standpoint of heteroecism and biologic specialization.

Various workers have reported biologic specialization in the sunflower rust, *Puccinia helianthi* Schw., but results have not been conclusive until 1922, when Bailey (3) was able to demonstrate definitely three distinct types of infection on *Helianthus annuus*, supplying final proof of the existence of biologic forms of this fungus.

Thus it is seen that a large number of rust species have been investigated from the standpoint of specialization to particular hosts, and the phenomenon has been found to be of wide occurrence.

Importance of the Present Investigation

The black stem rust is the greatest source of loss to the wheat-growing industry in Western Canada. So serious is the condition that wheat-growing is no longer profitable in large areas of the Red River Valley, and large numbers of farmers are threatening to abandon their farms unless a solution of the problem is forthcoming. Taking Canada as a whole, the losses in a normal year amount to from 5 per cent. to 10 per cent. of the wheat crop. This represents a loss of about eight million bushels in the prairie provinces alone, a loss which in epidemic years may be increased to seventy-five or one hundred million bushels.

Every wheat grower realizes his great annual loss, but he is powerless to prevent it. The weapons of the fruit grower, spraying and dusting, cannot be employed, not only on accouut of their prohibitive cost, but also because of the mechanical injury spraying machinery would cause to the maturing wheat crop, as rust does not appear on the plants until the heads are well advanced.

The farmers' only hope lies, then, in the production of rust-resistant varieties of wheat. Not only would such wheats largely eliminate one of the most important causes of unfavourable fluctuation in yield, thus greatly increasing the financial returns, but they would also contribute in an important degree to the safety and stability of the wheat-growing industry.

The problem is, however, complicated by the discovery of the occurrence of more than one biologic form of stem rust. This discovery was not only of scientific interest but had a direct bearing on the breeding of grain for rust resistance. It showed why "few varieties seem to be universally resistant" (Freeman and Johnson, 24), and explained the diverse opinions of workers in different localities as to the relative rust resistance of certain wheat varieties.

A study of biologic forms must precede the breeding of rust-resistant wheats. As Stakman has pointed out, "methods of breeding for rust resistance must be changed fundamentally. The breeder must know and work with those forms of rust which occur in the region for which his new variety is intended, and even then breeding must be very largely a regional or even a local problem."

With the numerous forms of stem rust on wheat present in the United States, the question naturally arose, "Did biologic forms of stem rust occur in Canada?" Until the present investigation was undertaken, no specific work had been done along these lines, although observations made by Dr. W. P. Thompson of Saskatchewan

University, in his breeding experiments, had suggested very strongly that such strains did exist.

The task of the Canadian plant pathologist, then, is to gain a definite knowledge of the number, characteristics, and geographical distribution of the biologic forms in Canada. It was with this end in view that this investigation was undertaken. Three years' results are now available and are presented in the following pages. It is hoped that they may be of immediate practical assistance to plant breeders.

Experimental Materials and Methods

Collections of rust were made in the field from the time the first pustules appeared in early summer until late in September. It was thought that if the observation were correct, that rust moves northward in waves across the continent, then possibly different biologic forms of rust might appear at different periods of the summer.

Material was collected in 81 representative localities of Manitoba, Saskatchewan and Alberta.

All the preliminary work of differentiating the numerous rust collections into distinct forms was carried out in the greenhouse, where conditions could be more definitely controlled than in the field.

Differential Hosts

The identification of the biologic forms necessarily involved the use of many varieties of wheat as differential hosts. Preliminary experiments with a number of different groups of wheat varieties indicated that the series used for this purpose by Stakman and his co-workers at the University of Minnesota were by far the most satisfactory. The list which this series comprises is given in Table I. The name of the variety is preceded in each case by the abbreviation used in the key and in the tables presented later.

Table I.—List of differential hosts used in identifying biologic forms.

Triticum aestivum

KRd—Kanred, C. I. 5146 (Kans. 2401).
Ko—Kota, C. I. 5878.
Ma—Marquis, C. I. 3641 (Minn. 1239).

Triticum compactum

Lc—Little Club, C. I. 4066.

Triticum dicoccum

Em—Emmer, C. I. 3686 (Minn. 1165).
Kpl—Khapli, C. I. 4013.

Triticum durum
Ac—Acme, C. I. 5284 (S.D. 284).
Arn—Arnautka, C. I. 4072 (S.D. 150).
Mnd—Mindum, C. I. 5296 (Minn. 470).
Spm—Speltz Marz, C. I. (Minn. 337).
Triticum monococcum
Enk—Einkorn, C. I. 2433.
C. I. = U.S. Dept. Agr. Cereal Investigation Office number.

Table II.—Explanation of symbols used to indicate types and degrees of infection of wheat varieties by *Puccinia graminis*.

0. Immune.
No uredinia developed; hypersensitive (sharply chlorotic) flecks sometimes present.
1. Very Resistant.
Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive areas.
2. Moderately Resistant.
Uredinia isolated and small to medium in size; hypersensitive areas present; pustules often surrounded by green islands.
3. Moderately Susceptible.
Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
4. Very Susceptible.
Uredinia large, numerous and confluent; hypersensitiveness entirely absent.

Miscellaneous Symbols.
(;) Chlorotic flecks.
(x) Mixture of strains.

Any particular collection usually comprised a mixture of forms which first had to be separated by cultural experiments. Each form was then cultured separately upon all the differential hosts, and this operation was repeated until constant results were obtained. The reactions towards all of the wheats were then compared with the reactions of the forms described by Stakman and Levine. In all cases they were found to coincide with the reactions of one or other of the forms described by these authors, and consequently the same strain numbers were adopted.

The methods of inoculating and culturing the rusts were similar to those described by Stakman and Piemeisel (59). In recording the

ANALYTICAL KEY TO BIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI

Ma - R
- Krd - R
  - Ko - R
    - Arn - R
      - Kub - R.....11
      - Kub - S
        - Enk - R.....XXV11
        - Enk - S.....XX111
    - Arn - S
      - Mnd - R.....V1
      - Mnd - S
        - Kub - R.....1V
        - Kub - S
          - Enk - R.....XV1
          - Enk - S.....X1V
  - Ko - S
    - Mnd - R.....XXV111
    - Mnd - S.....X1X
- Krd - S
  - Arn- R.....V11
  - Arn- S.....X

Ma - S
- Krd - R
  - Ko - R.....XX1V
  - Ko - S.
    - Arn - R.....1
    - Arn - X
      - Em - R.....XX1X
      - Em - S..... XXX
    - Arn - S
      - Mnd - R.....XXV1
      - Mnd - S
        - Kub - R
          - Em - R.....V
          - Em - S.....V111
        - Kub - S
          - Enk - R.....XX1
          - Enk - S
            - Em - R.....XV11
            - Em - S.....1X
- Krd - S
  - Ko - R.....XXX1
  - Ko - S
    - Arn - R
      - Mnd - R
        - Kub - R.....111
        - Kub - S
          - Ao - R.....XX
          - Ao - S.....XV111
      - Mnd - S
        - SpM - R.....XXV
        - SpM - S.....XX11
    - Arn - X.....XXX11
    - Arn - S
      - Mnd - R.....X11
      - Mnd - S
        - Kub - R.....X111
        - Kub - S
          - Em -R.....X1
          - Em -S.....XV

type and degree of infection the symbols adopted by Stakman and Levine in their work on biologic specialization were used. A detailed explanation of the meaning of these is given in Table II. Briefly stated, 0, 1 and 2 designate varying degrees of resistance, collectively referred to by the symbol R in the "Key to Biologic Forms" given above. On the other hand, 3 and 4 designate degrees of susceptibility, referred to in the key by the symbol S. Experience has shown that the degree of resistance or susceptibility of a given wheat variety to a given biologic form, under greenhouse conditions, is remarkably constant. In the field, however, there is a tendency towards a somewhat lighter degree of infection. In some cases, a variety that would ordinarily score 3 in the greenhouse may score 2 in the field. Varieties are given the score representing the degree of infection to which they most nearly approximate, this figure being followed, where necessary, by *plus* or *minus* signs to indicate deviations from the standard.

The procedure just outlined for identification of the forms was facilitated by the use of the accompanying key devised by Stakman and Levine. This key is constructed upon the same principle as an ordinary botanical key. The compound symbols employed consist of the abbreviated designations for the differential hosts, followed by R or S, denoting resistant and susceptible, respectively. The use of the key will be illustrated by the following example: A form collected on Kanred at Saskatoon, Saskatchewan, on September 14, 1920, was cultured on the hosts given in Table I. Marquis proved susceptible. This threw the form in question into the second main section of the key, viz., Ma—S. The test on Kanred showed this variety also to be susceptible. This led us to section KRd—S of the key. Kota also proved susceptible. This placed the form in Ko—S. As Arnautka and Mindum were both resistant, we arrived at Mnd—R. Kubanka, on the other hand, was susceptible, thus bringing up to Kub—S. But one variety, Acme, remained to be examined. Since this proved susceptible the form was identified as number XVIII.

Results

Fourteen biologic forms have been demonstrated, by the methods described, to be present in Canada. These forms have proved to be identical with some of those described by Stakman in the United States. In the latter country, however, a considerable number of additional forms have been demonstrated.[1] The date and place of collection, host on which collected, and complete infectional characterization of the Canadian forms are summarized in Table III.

[1]Unpublished data from E. C. Stakman.

TABLE III

DISTRIBUTION IN CANADA OF THE BIOLOGIC FORMS OF *Puccinia graminis tritici* WITH A RECORD OF THEIR INFECTION CAPABILITIES

Form	Place of collection of rust	Date	Host on which collected	Character of infection on differential hosts											
				Lc	Ma	KRd	Ko	Arn	Mnd	Spm	Kub	Ac	Enk	Em	Kpl
I	Brandon, Man. Carnoustie, Sask. Morris, Man.	Sept., 1919 Sept., 1919 Aug, 23, 1920	Marquis Marquis Marquis	4	4	0	3+	0;	1=	1−	3±	3	3	0;	1.
III	Brandon, Man. Morden, Man. Rosthern, Sask. Watrous, Sask.	July 27, 1920 " " Sept. 2, 1920 Sept. 13, 1920	Marquis Ruby Minister Marquis	4	3++	3++	3+	1=	0	1−	1	3	3	1	0;
IX	Brandon, Man. Carlyle, Sask. Edmonton, Alta. Leslie, Sask. Rosthern, Sask. Saskatoon, Sask. Winnipeg, Man.	Aug. 28, 1920 Sept. 22, 1920 Sept. 8, 1920 Sept., 1919 Sept. 2, 1920 Sept. 25, 1920 Aug. 25, 1920	Emmer Marquis Emmer Marquis Minister Marquis Emmer	4	3	0	3±	3=	3++	3+	3++	3−	3+	3±	0.
XI	Brandon, Man. Edmonton, Alta. Moose Jaw, Sask.	Sept., 1919 Sept., 1919 Aug. 5, 1920	Marquis Marquis Marquis	4	3++	3++	3+	4	4	4	4	3+	3	0;	1−
XII	Indian Head, Sask. Moose Jaw, Sask.	Aug. 30, 1920 Aug. 5, 1920	Marquis Marquis	4	4	3	4−	3	1	1	1+	4−	3+	1	1
XV	Melfort, Sask.	Sept., 1919	Marquis	4	4	4	4=	4	4−	4−	4−	4=	3±	4−	1=

XVII	Alameda, Sask.	Sept. 28, 1921	Marquis												
	Camrose, Alta.	Sept. 10, 1920	Marquis	4	4	0	4=	4±	4	4−	4	3	3+	0;	1=
	Carleton, Sask.	Sept., 1919	Marquis												
	Dauphin, Man.	Sept. 1, 1920	Marquis												
	Edmonton, Alta.	Sept. 8, 1920	Emmer												
	Govan, Sask.	Sept. 15, 1920	Marquis												
	Howell, Sask.	Sept., 1919	Marquis												
	Indian Head, Sask.	July 14, 1920	Ruby												
	" " "	July 19, 1920	Ruby												
	Morris, Man.	July 23, 1920	Marquis												
	Napinka, Man.	Sept. 24, 1920	Marquis												
	Prince Albert, Sask.	Sept., 1919	Marquis												
	Rapid City, Man.	July 16, 1920	Marquis												
	Red Berry Lake, Sask.	Sept., 1919	Marquis												
	Rosthern, Sask.	Sept. 2, 1920	Minister												
	Saltcoats, Sask.	Sept., 1919	Marquis												
	Saskatoon, Sask.	Aug. 2, 1920	Ruby												
	"	Sept., 1919	Marquis												
	Shellbrook, Sask.	Aug. 16, 1920	Marquis												
	Stettler, Atla.	Sept. 9, 1920	Marquis												
	"	"	Hordeum jubatum												
	Vermilion, Alta.	Sept., 1919	Marquis												
	Winnipeg, Man.	July 5, 1920	Ruby												
	"	July 17, 1921	Marquis												
	Yorkton, Sask.	July 15, 1921	Marquis												
	Zealandia, Sask.	Sept., 1919	Marquis												
XVIII	Melfort, Sask.	Sept., 1919	Marquis	4	4	3+	3−	0;	0;	2=	3	3	3	0;	0;
	Riverstone, Sask.	Sept., 1919	Marquis												
	Saskatoon, Sask.	Sept. 1, 1920	Kanred												
	Vermilion, Alta.	Sept. 11, 1920	Marquis												
	Weyburn, Sask.	July 9, 1920	Marquis												
	Yorkton, Sask.	July 15, 1920	Marquis												

Form	Place of collection of rust	Date	Host on which collected	Character of infection on differential hosts											
				Lc	Ma	KRd	Ko	Arn	Mnd	SpM	Kub	Ac	Enk	Em	Kpl
XIX	Indian Head, Sask.	Sept., 1919	Marquis	4	1++	0	3=	4−	3+	3+	4±	4−	4−	0	1.
XXI	Alameda, Sask.	Sept. 18, 1921	Marquis												
	Carleton, Sask.	Sept., 1919	Marquis	4	4	0;	3++	4	3++	3+	3+	3	1=	0	0.
	Winnipeg, Man.	Sept., 1919	Marquis												
XXIV	Carnoustie, Sask.	Sept., 1919	Marquis	4	3+	0;	1±	3++	4	4	4	4	3	0;	0.
	Saskatoon, Sask.	July 17, 1921	Marquis												
XXIX	Brandon, Man.	July 27, 1920	Marquis	4	4	0	3+	x	x	x	x	3±	3+	0;	1.
	"	Sept. 27, 1920	Marquis												
	Carlyle, Sask.	Sept. 9, 1920	Marquis												
	Edmonton, Alta.	Aug. 12, 1920	Club												
	Elbow, Sask.	Sept. 10, 1920	Marquis												
	Indian Head, Sask.	Aug. 30, 1920	Kota												
	Lacombe, Alta.	Sept. 9, 1920	Barley												
	Macleod, Alta.	Sept. 22, 1920	Marquis												
	Mervin, Sask.	Sept. 9, 1920	Marquis												
	Moose Jaw, Sask.	Sept. 20, 1920	Marquis												
	Morden, Man.	Aug. 4, 1921	Marquis												
	Prince Albert, Sask.	Aug, 2, 1920	Marquis												
	Quill Lake, Sask.	Sept., 1919	Marquis												
	Rosetown, Sask.	Sept. 12, 1920	Marquis												
	Rosthern, Sask.	Sept. 2, 1920	Pentad												
	Treesbank, Man.	Aug. 3, 1920	Marquis												
	Vegreville, Man.	Sept. 11, 1920	Hordeum jubatum												

	Vegreville, Man.	Sept. 9, 1920	Marquis													
	Weyburn, Sask.	Sept. 21, 1920	Marquis													
XXX	Carlyle, Sask.	Sept. 22, 1920	Marquis	4	4+	0;	3+	x	x	x		x	3	3±	4−	0;
XXXII	Morden, Man.	July 27, 1920	Ruby	4+	3+	4	3	x	x	x		x	3	3	1=	1−
	Rosthern, Sask.	Sept. 2, 1920	Barley													
	Treesbank, Man.	Aug. 3, 1920	Ruby													
	Vermilion, Alta.	Sept. 11, 1920	Marquis													
	Winnipeg, Man.	July 17, 1921	Marquis													

Identity and Nature of the Forms Isolated

The identity and nature of the forms isolated can be seen from Table III, which includes a summary of the infection reactions of each form on all the differential hosts. In order to illustrate the use of this tabular summary, the data for two forms have been abstracted from the table, and are given below, together with a summarized description in words corresponding to the symbols used in the table.

Character of infection on differential hosts

Form	Lc	Ma	KRd	Ko	Arn	Mnd	SpM	Kub	Ac	Enk	Em	Kpl
I	4	4	0	3+	0;	1−	1−	3±	3	3	0;	1
XV	4	4	4	4=	4	4−	4−	4−	4=	3−	4−	1=

Little Club	I	Heavy normal infection.
	XV	Heavy normal infection.
Marquis	I	Heavy normal infection.
	XV	Heavy normal infection.
Kanred	I	Absolute immunity.
	XV	Heavy normal infection.
Kota	I	Moderate susceptibility.
	XV	Moderate susceptibility.
Arnautka	I	Absolute immunity, chlorotic flecks.
	XV	Heavy normal infection.
Mindum	I	Decided resistance.
	XV	Heavy infection.
Speltz Marz	I	Decided resistance
	XV	Heavy infection.
Kubanka	I	Moderate susceptibility.
	XV	Heavy infection.
Acme	I	Moderate susceptibility.
	XV	Moderate susceptibility.
Einkorn	I	Moderate susceptibility.
	XV	Moderate susceptibility.
Emmer	I	Absolute immunity, chlorotic flecks.
	XV	Heavy infection.
Khapli	I	Very high resistance.
	XV	Decided resistance.

As will be seen in Table III, most of the forms were isolated and identified several times from material of different collections. Since the procedure was in principle the same in all cases it will be sufficient to present here the records of one typical series of inoculations for each form. These are given in Diagrams 1 to 14. An explanation of the symbols used will be found under Diagram 1.

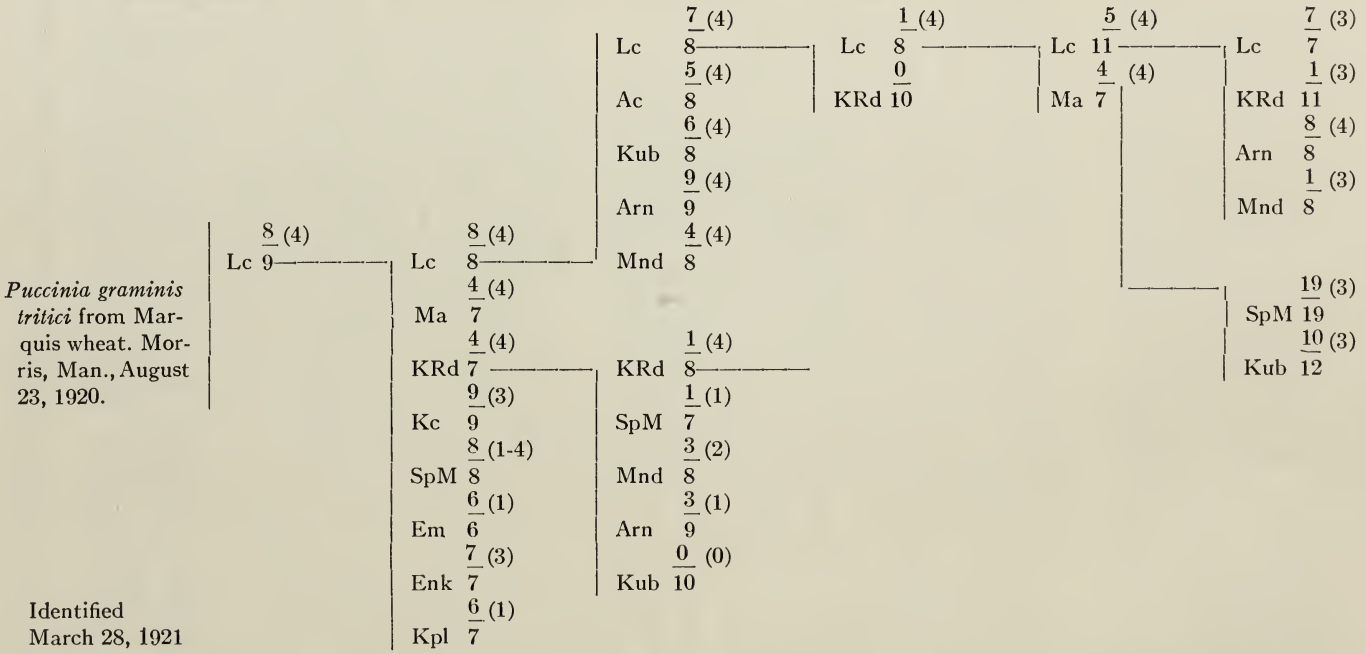
In Diagrams 3, 4, 5, 7, 9, 10, 11, and 12, it will be seen that the rust collections in question proved to be composed of but one form. It should be pointed out, however, that these cases have been deliberately selected to simplify the presentation of these illustrative data; the circumstance of a collection consisting of only one form was unusual. The average condition is better represented in Diagrams 1, 6, 8, 13, and 14, where the collections consisted of 2 forms. Occasionally a collection yielded 3 forms, as in the case shown in Diagram 2.

It will be seen in the latter group of diagrams that the transference of the rust of a given collection to the test wheats frequently resulted in the appearance of 2 forms of pustules, small and large, a "1-4" infection. It was then assumed that a mixture of forms was present, resistance of the host to one form being represented by "1" and susceptibility to the other form by "4." The next step was to separate the forms. Accordingly spores from the small and large pustules were transferred separately to different plants of the same host variety. If practically all the pustules resulting from this inoculation were small in the case of the one host plant, and large in the case of the other, the presence of at least 2 forms in the original rust collection was practically confirmed. These results were always checked by further inoculations on all the differential hosts given in the key, a procedure which, as previously noted, served also for the final identification of the forms.

The reactions of Forms XXIX, XXX and XXXII are still imperfectly understood. They always give two degrees of infection, "1-4," on the Arnautka, Mindum, Speltz Marz and Kubanka varieties of durum wheat; whereas, on all the remaining differential hosts they behave normally as pure forms. For two years every effort has been made to separate these apparent mixtures, but in vain. Cultures made from a single spore of each form still failed to resolve the components.[2] It has been suggested that these forms must be heterozygous, or perhaps homozygous with a genetic composition resulting in this type of infection. It seems to the writer at least equally possible that the aberrant behaviour is connected with the physiological relationship of the fungus and the host, perhaps even arising from the genetic composition of the host itself with regard to rust resistance. However, the problem must await further investigation, and perhaps the development of more refined technique, before it can be explained.

[2]Unpublished data from E. C. Stakman.

DIAGRAM I—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORMS I AND XVII



Explanatory Note:—The fractions following the abbreviations of varietal names indicate the number of leaves inoculated (denominator) and the number developing consequent uredinia (numerator). The figures in brackets indicate the degree of infection. (Cf. Table II).

DIAGRAM 2.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORMS III, IX AND XVII

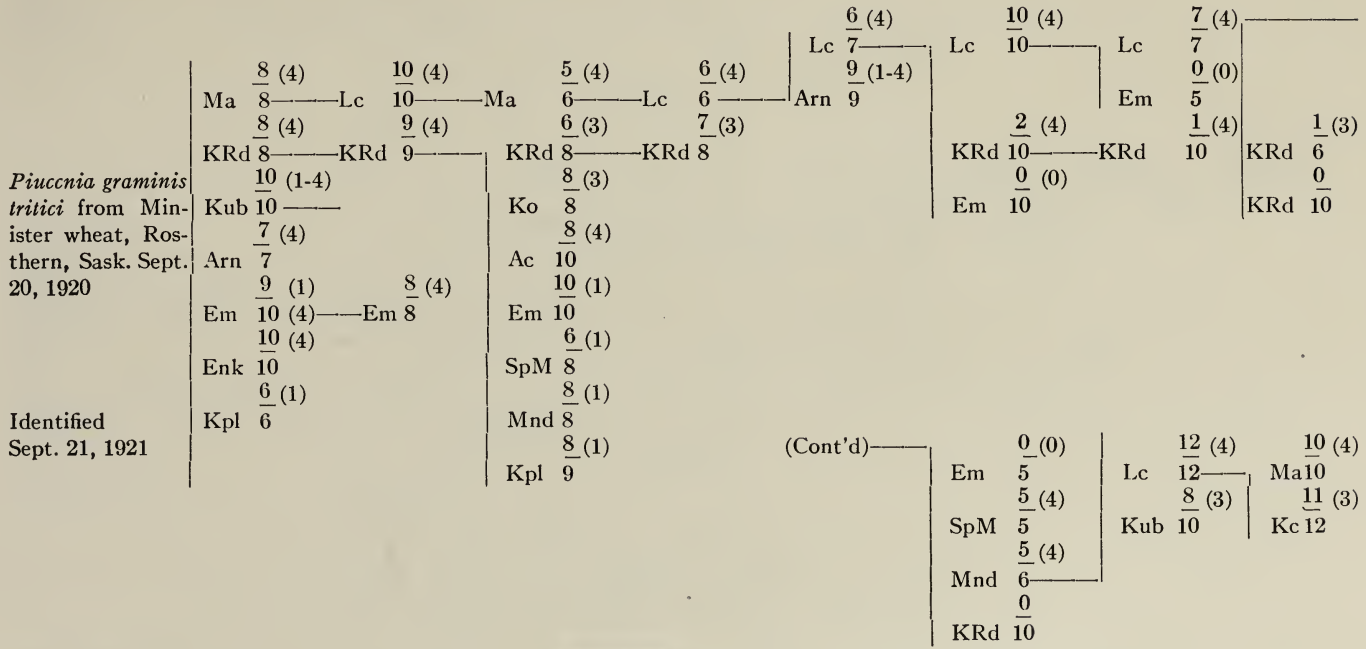


DIAGRAM 3.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM IX

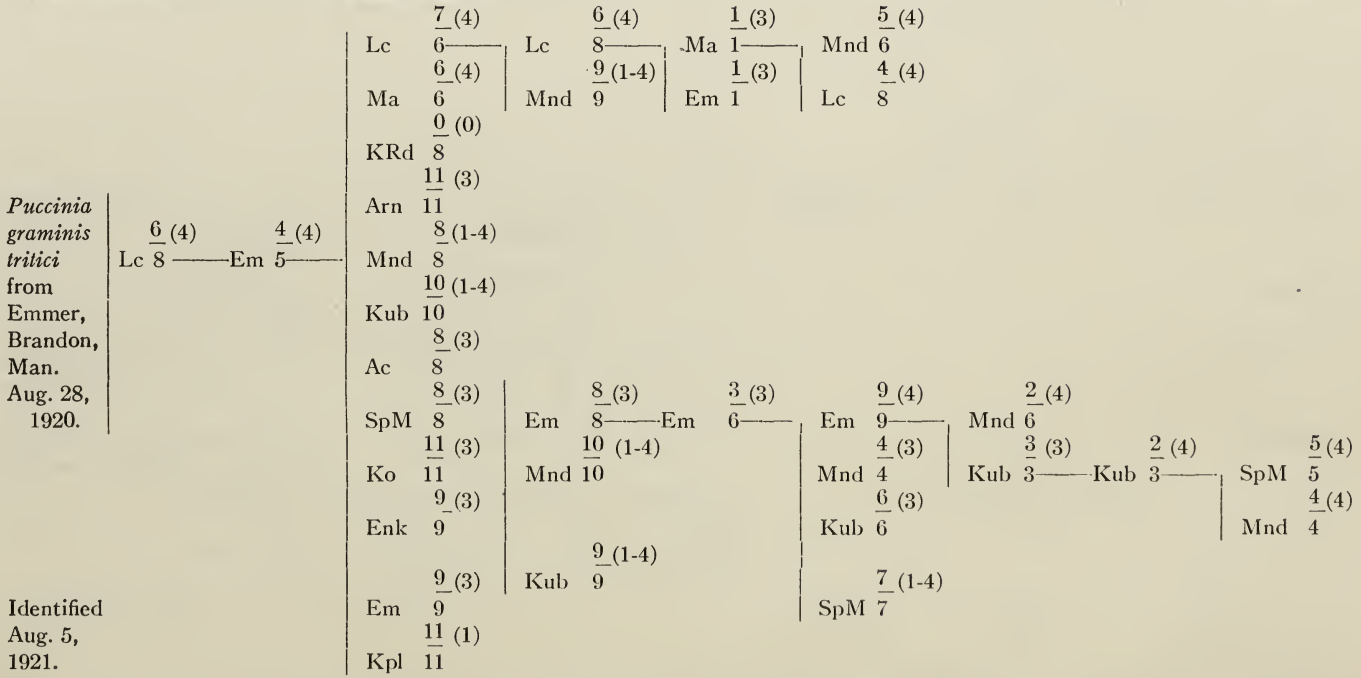


DIAGRAM 4.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XI

Puccinia graminis tritici
From Marquis wheat, Edmonton, Alta.
September 15, 1919.

Identified
August 20, 1920

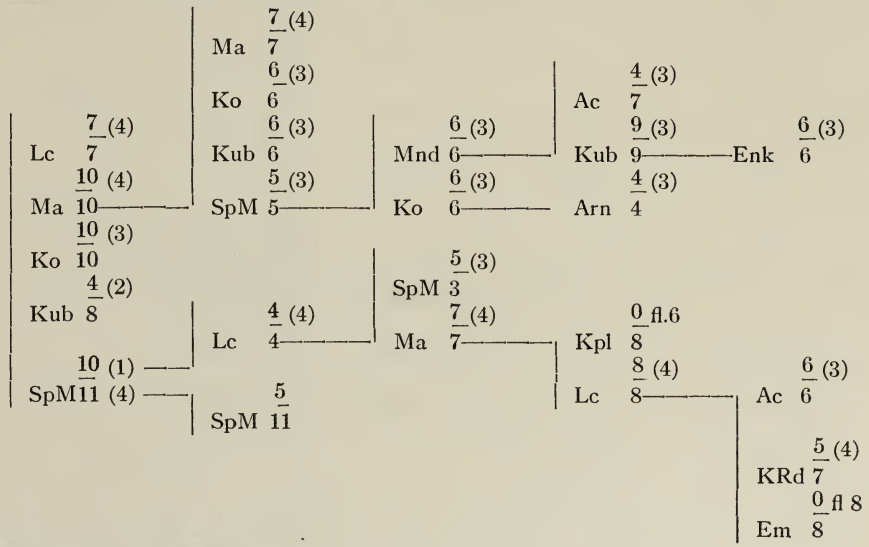


DIAGRAM 5.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XII

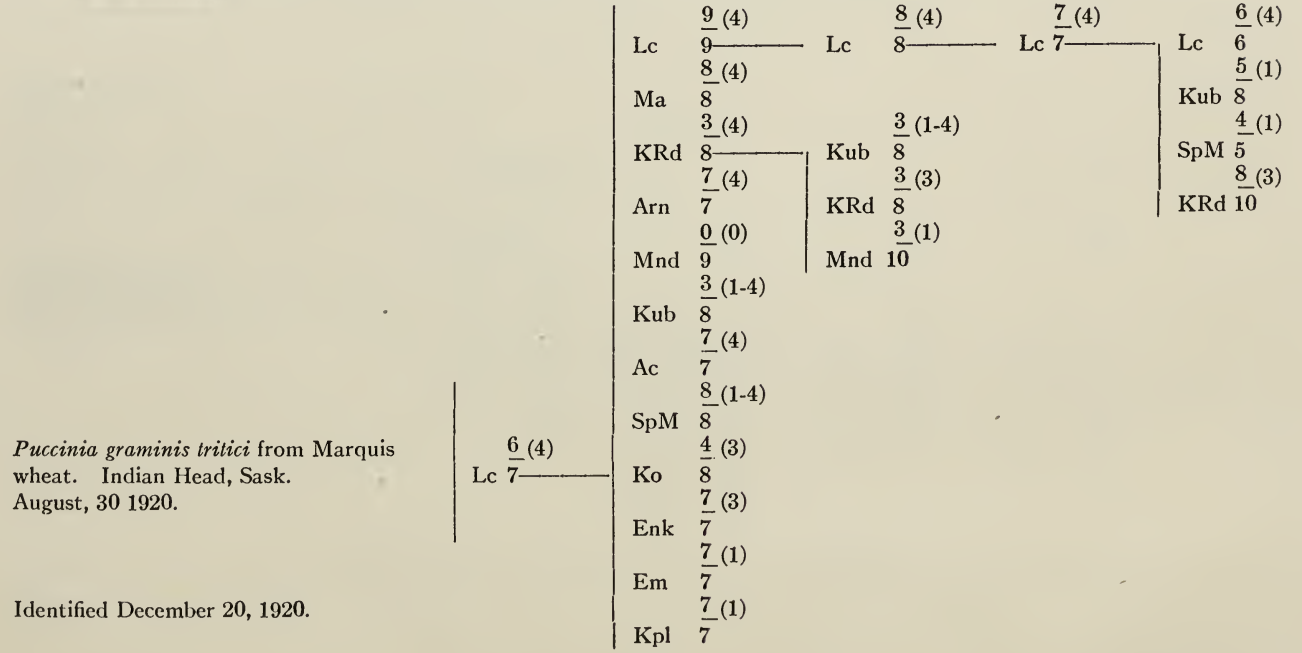
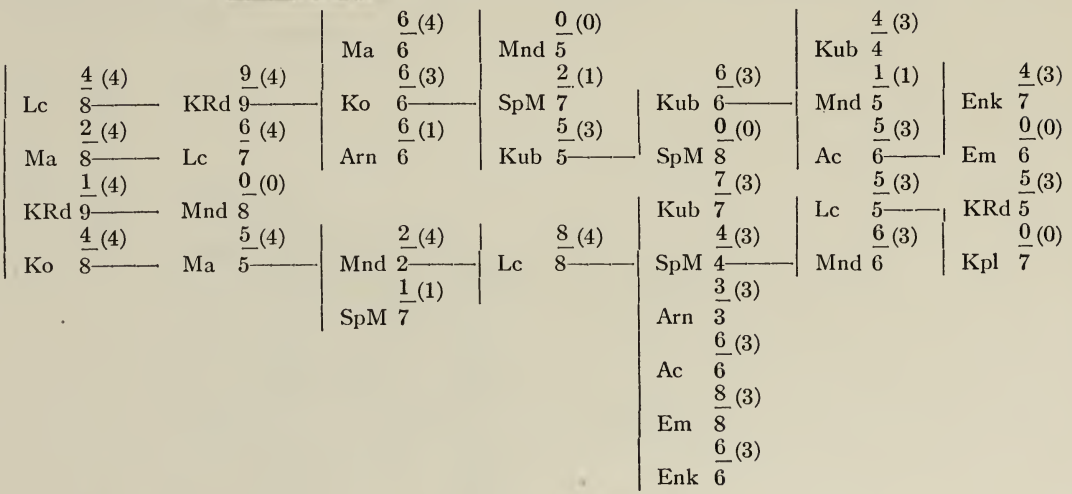


DIAGRAM 6.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORMS XV AND XVIII

Puccinia graminis tritici from Marquis wheat. Melfort, Sask. September 15, 1919.



Identified August 6, 1920.

DIAGRAM 7.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XVII

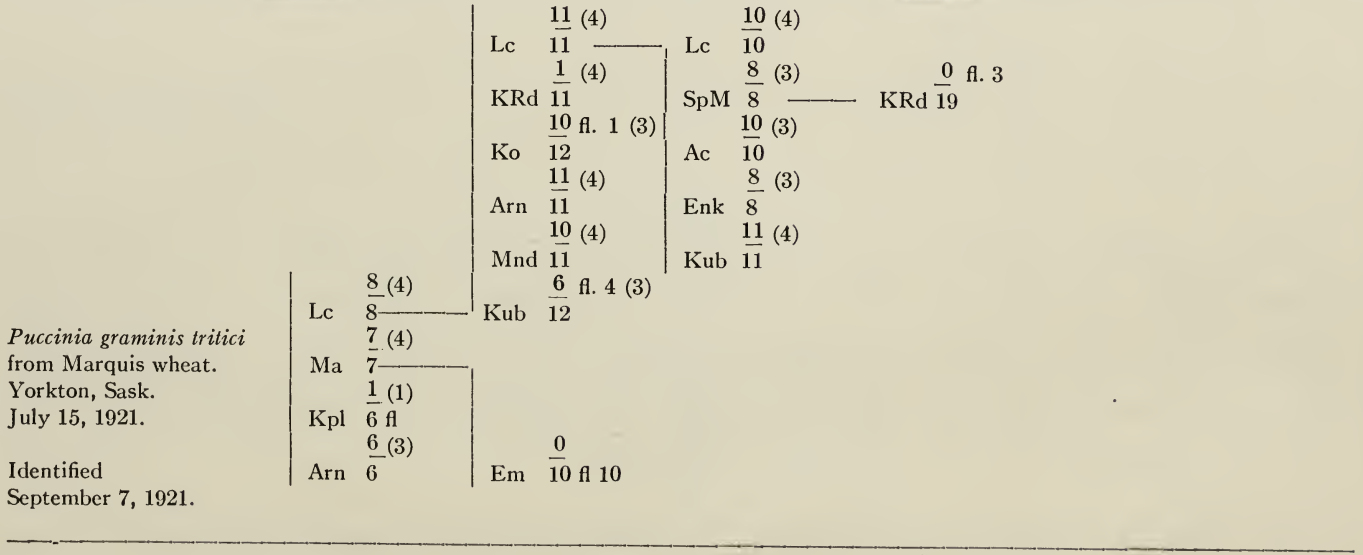


DIAGRAM 8.—A SERIES OF INOCULATION RESULTING IN THE IDENTIFICATION OF FORM XVIII

(See Diagrams 6 and 14.)

DIAGRAM 9.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XIX

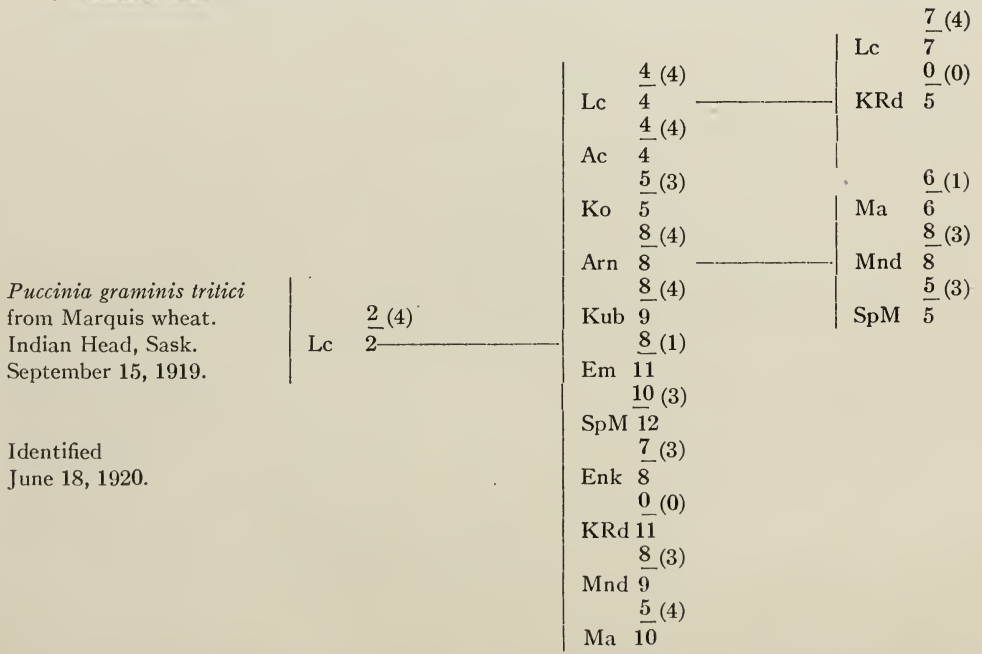


DIAGRAM 10.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XXI

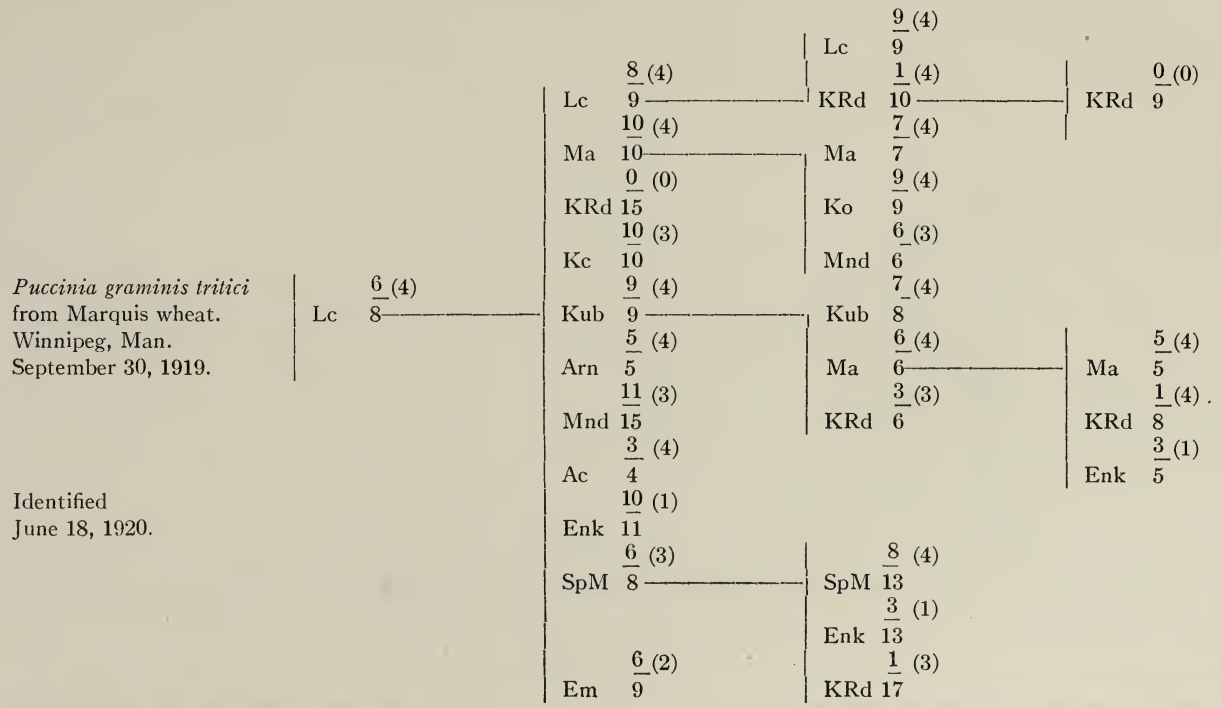


DIAGRAM 11.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XXIV

Puccinia graminis tritici from Marquis wheat.
Saskatoon, Sask.
July 17, 1921.

Identified September 7, 1921.

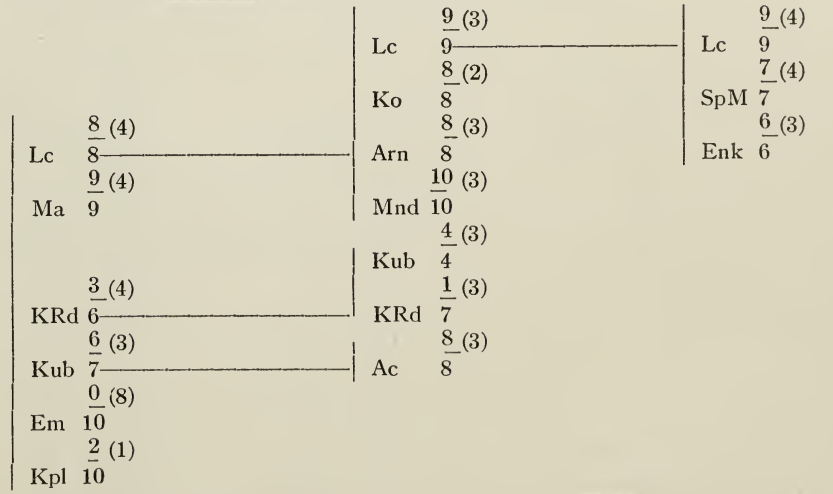


DIAGRAM 12.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORM XXIX

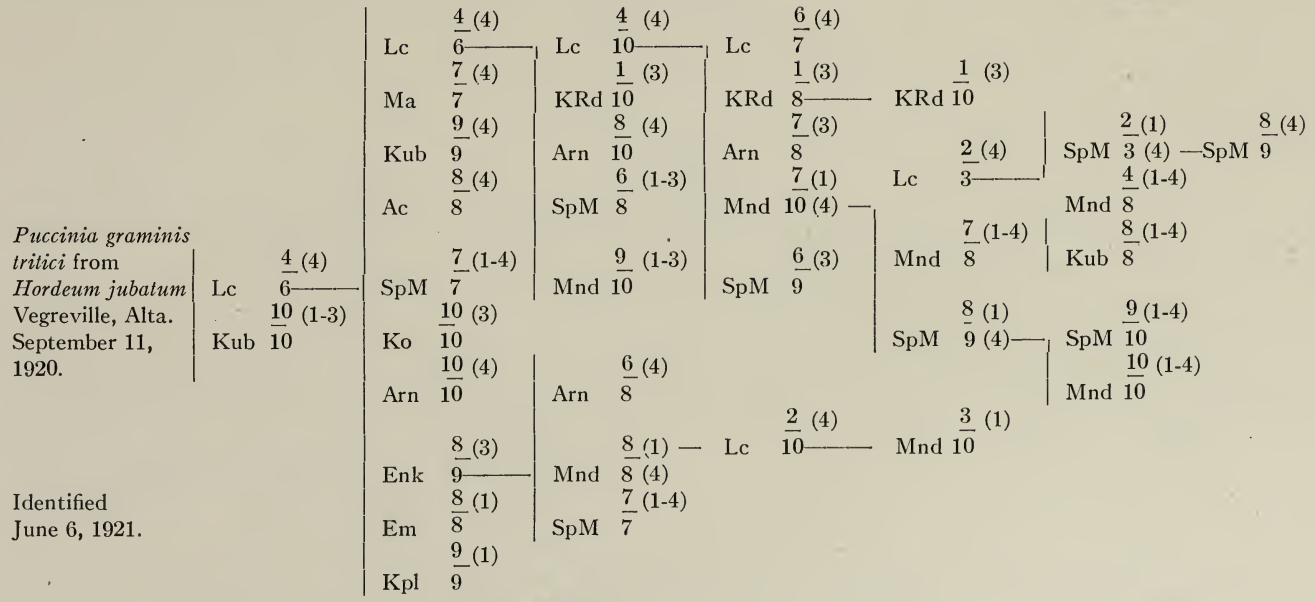


DIAGRAM 13.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORMS XXX AND IX

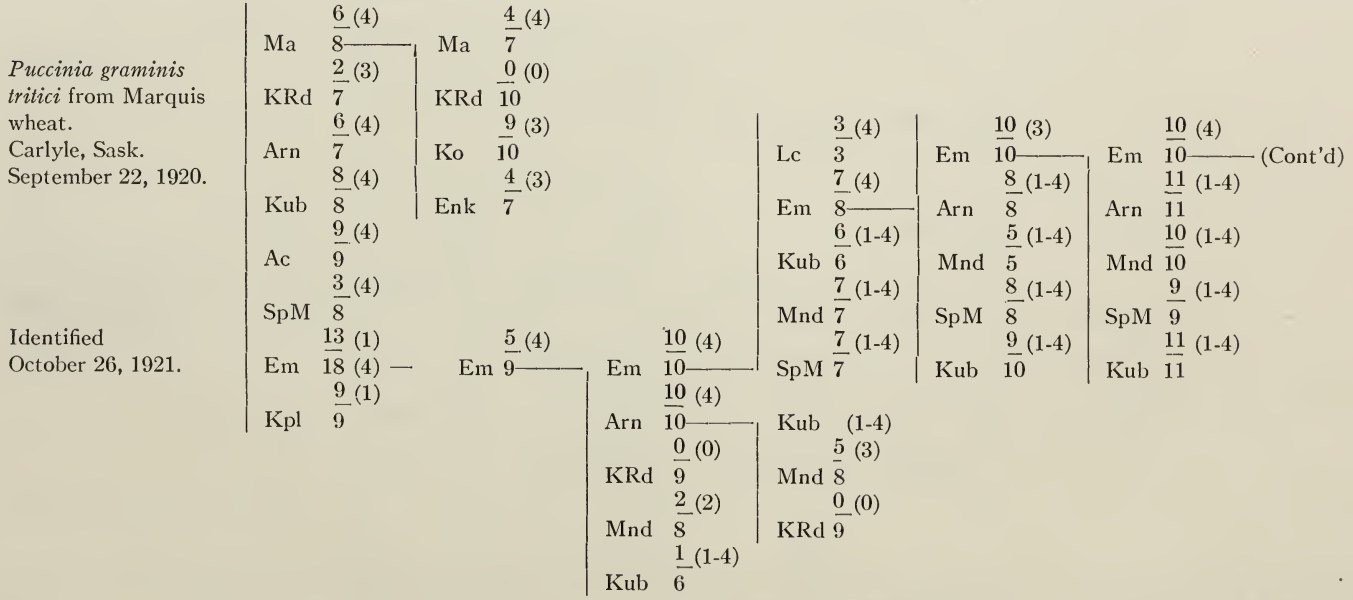


DIAGRAM 13 (*Continued*)

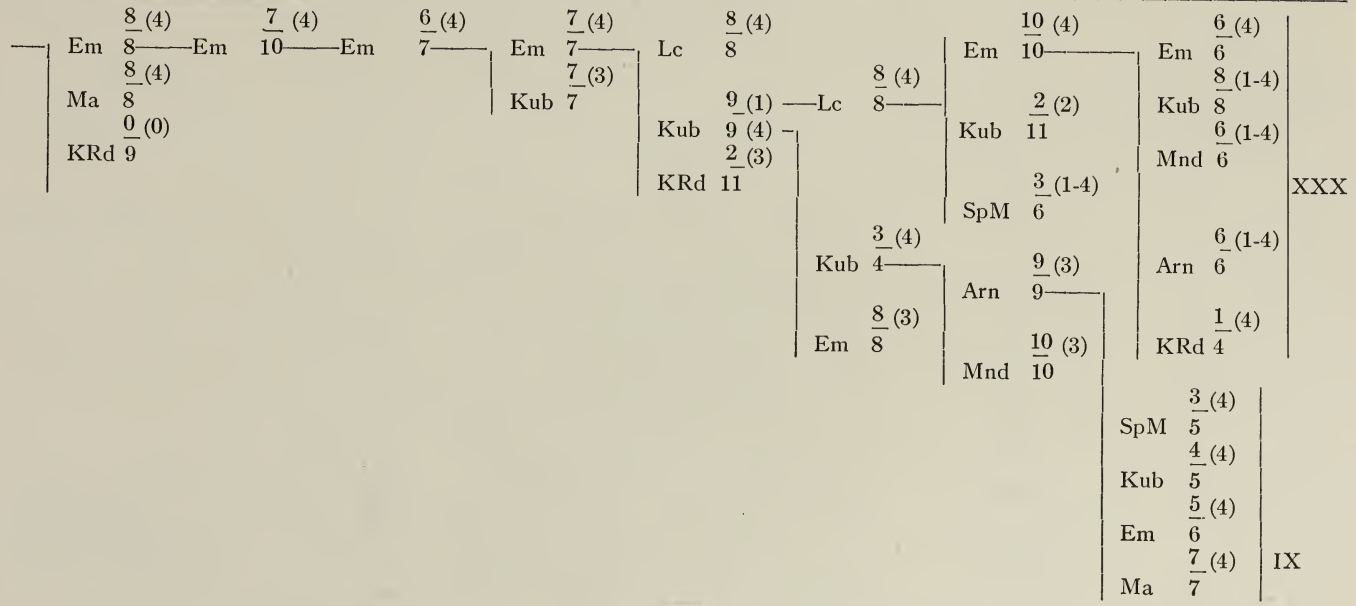


DIAGRAM 14.—A SERIES OF INOCULATIONS RESULTING IN THE IDENTIFICATION OF FORMS XVIII AND XXXII

Puccinia graminis tritici
from Marquis wheat.
Vermilion, Alta.
September 11, 1920.

Identified
May 21, 1921.

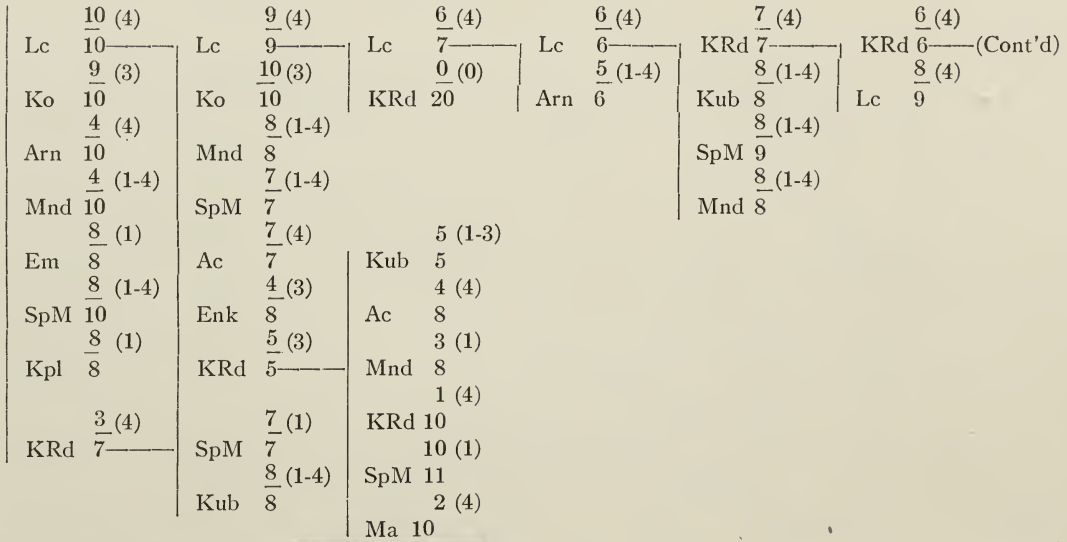
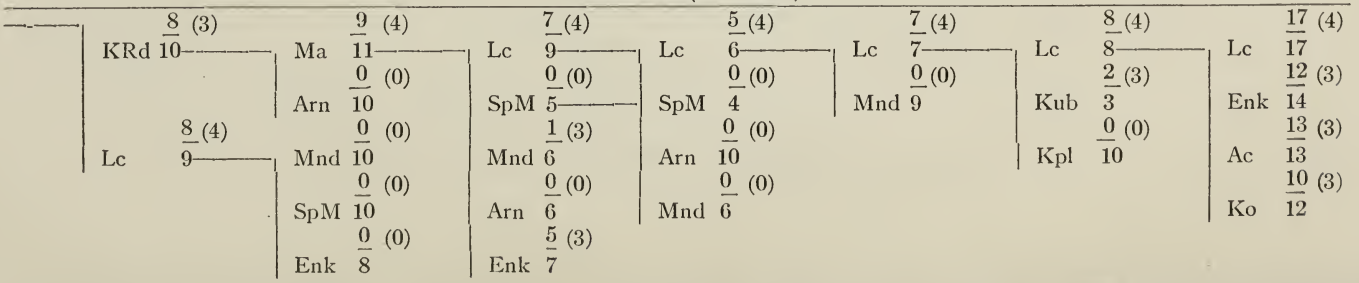


DIAGRAM 14 (*Continued*)



Attention should be drawn to two other rather striking facts, evident in the diagrams. It will be seen that Khapli, an emmer from India, is resistant to every form of rust found, while Little Club is completely susceptible in all cases. From the standpoint of the plant breeder, it is unfortunate that Khapli is very difficult to hybridize successfully with the bread wheats.

Importance of Forms Isolated

The factors which determine the importance of a biologic form are its varietal range and virulence, and its distribution and frequency of occurrence.

Varietal Range and Virulence.

A glance at Table III will show that rusts from different localities vary greatly in their parasitic behaviour to wheat varieties. Marquis, a wheat quite susceptible to practically all forms of rust in Western Canada, is highly resistant to a form found at Indian Head. Four distinct biologic forms were found at Saskatoon; one of these infected White Spring emmer very heavily, while another scarcely infected it at all. In the same way, Kanred showed heavy normal infection at Brandon, Yorkton, Moose Jaw, and Edmonton, and complete immunity at Winnipeg, Prince Albert, and Lacombe. One of the forms, XV, was very virulent on all but one of the varieties inoculated, while another, III, was so weak that it could attack only a few varieties successfully. Several forms differed from one another only in their action on one or two varieties, but these differences were always definite and consistent.

Usually more than one biologic form was found on the same variety, sometimes even on the same plant. From one rusted plant collected at Rosthern, Saskatchewan, were isolated three distinct forms, III, IX, and XVII. On the other hand, the same form was present on a great variety of hosts and apparently was not changed in any way by association with this host. One form collected on barley, emmer, club wheat, and various other varieties of spring and winter wheats, as well as on wild grasses, gave the same reaction in all cases, whether taken from the wild grasses of northern Alberta or from the hard spring wheats of southern Manitoba. This constancy of behaviour will be more fully discussed in a later section of this paper.

Distribution and Frequency.

The geographical distribution of the various biologic forms is still imperfectly known. However, tentative maps have been prepared showing the areas in which the more frequently occurring forms have been collected. These have been made by the simple expedient of connecting with a broken line the more outlying points at which collections have been made. The boundaries so arrived at will, no doubt, be extended by further exploration. Indeed, it seems probable that no southern boundary exists, and that in some cases, at least, the northern boundary may coincide with the limits of the wheat-growing area. On the other hand, it is probable that the frequency of occurrence will diminish towards the outlying parts of the areas involved, although, in special cases, natural barriers may possibly interpose an abrupt limit. It must be noted, further, that the date of occurrence in a given locality possibly varies with remoteness from the point of origin of the infection. This may explain why collections in the Calgary to Edmonton district of Alberta are rarely possible before September. Such conditions are, of course, extremely fortunate for the region concerned, since infection occurring thus late cannot do any serious damage to the crop.

In Fig. 1, A. is shown the area from which collections of a rather virulent form, XVII, have been made. This is by far the most widely distributed of all the forms isolated. It is found in 26 distantly separated districts of Manitoba, Saskatchewan, and Alberta, embracing a variety of climatic conditions, especially in regard to rainfall. As already pointed out, the area delimited on the map may safely be taken as a very conservative index of distribution; in many places outside this area, but one collection of rusted material has been made. The persistance of Form XVII is shown by the fact that once it has been found in any locality, collections in one or more succeeding seasons have rarely failed to demonstrate its recurrence.

Within the same area are included, of course, many other forms; eight have already been found.

In Fig. 1, B. is shown the area in which have been found 6 forms (I, IX, XVII, XXI, XXIX, XXX) all of which cause the same infection upon all the bread wheats, as may be seen by reference to Table III. That is not to say that these 6 forms are identical, since, as may also be seen, they vary greatly in their parasitism towards other varieties. The great importance of this group will be apparent when it is pointed out that they include 70 per cent. of the rust collections made, and cover practically the whole area now occupied

by the grain growing industry. In fact, this area will probably continue, for a long time, to be the main wheat centre. In portions of southern Alberta and Saskatchewan, on account of the rather arid conditions which prevail, wheat will probably never be the crop of first importance. With regard to the more northern and western districts it has been pointed out already that rust infection seldom

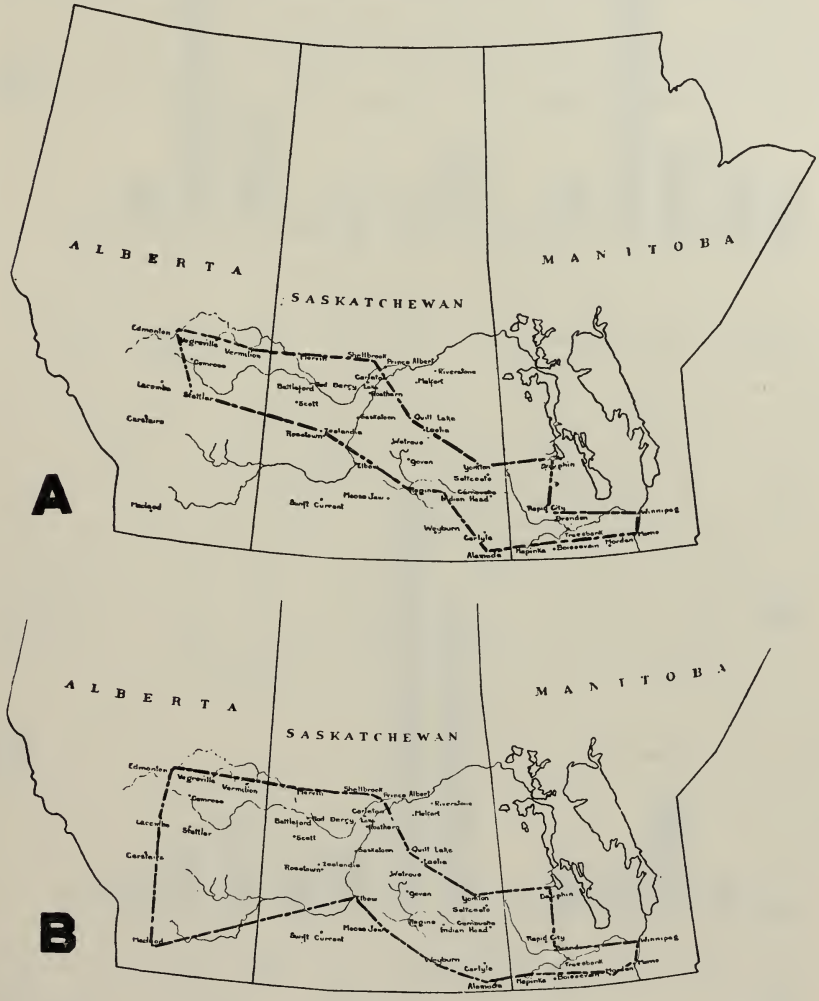


Fig. 1. A. Distribution of Form XVII (virulent) in area explored.
B. Area in which have been found the 6 dominant forms.

occurs early enough in the season to cause material damage. Obviously, the group of rust strains just considered should constitute the first point of attack for the plant breeders. The winning of this objective should go a long way towards the solution of the general problem.

The distribution of the 8 remaining forms is shown in Figs. 2, A and B. A key to the symbols employed is given in the lower right-hand corner. On the whole, these forms were rather widely scattered, although Form IX, which attacks emmer heavily, apparently was more prevalent in the eastern half of the wheat area than

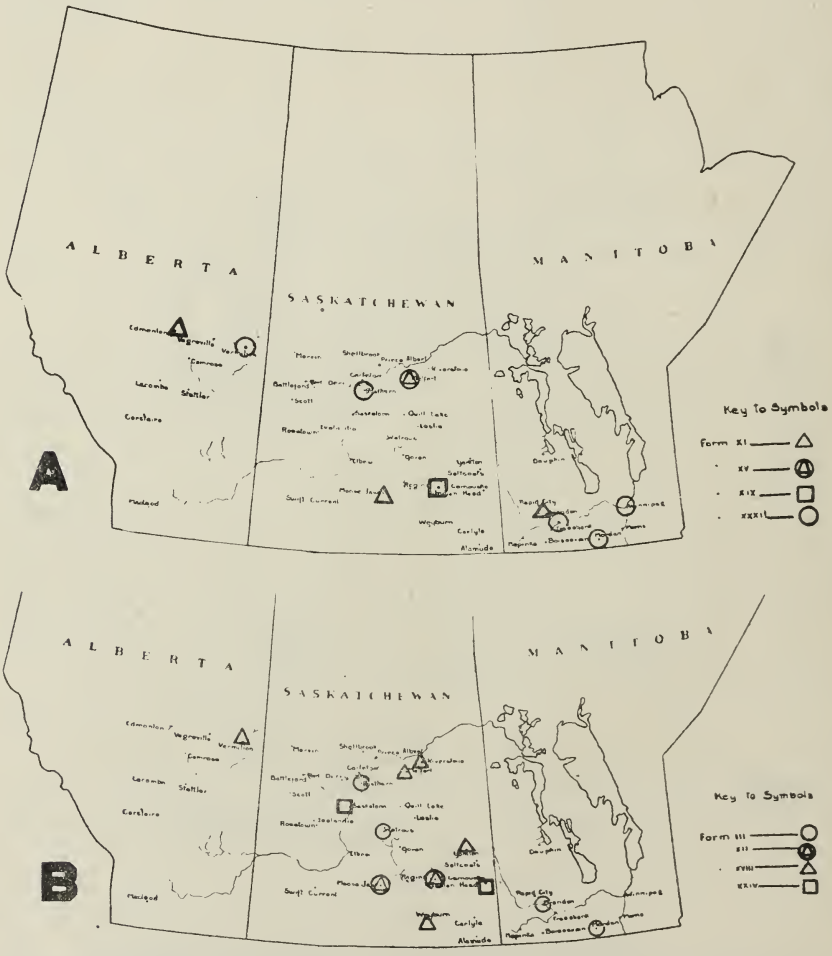


Fig. 2. Localities in which have been collected the forms not included in Fig. 1.

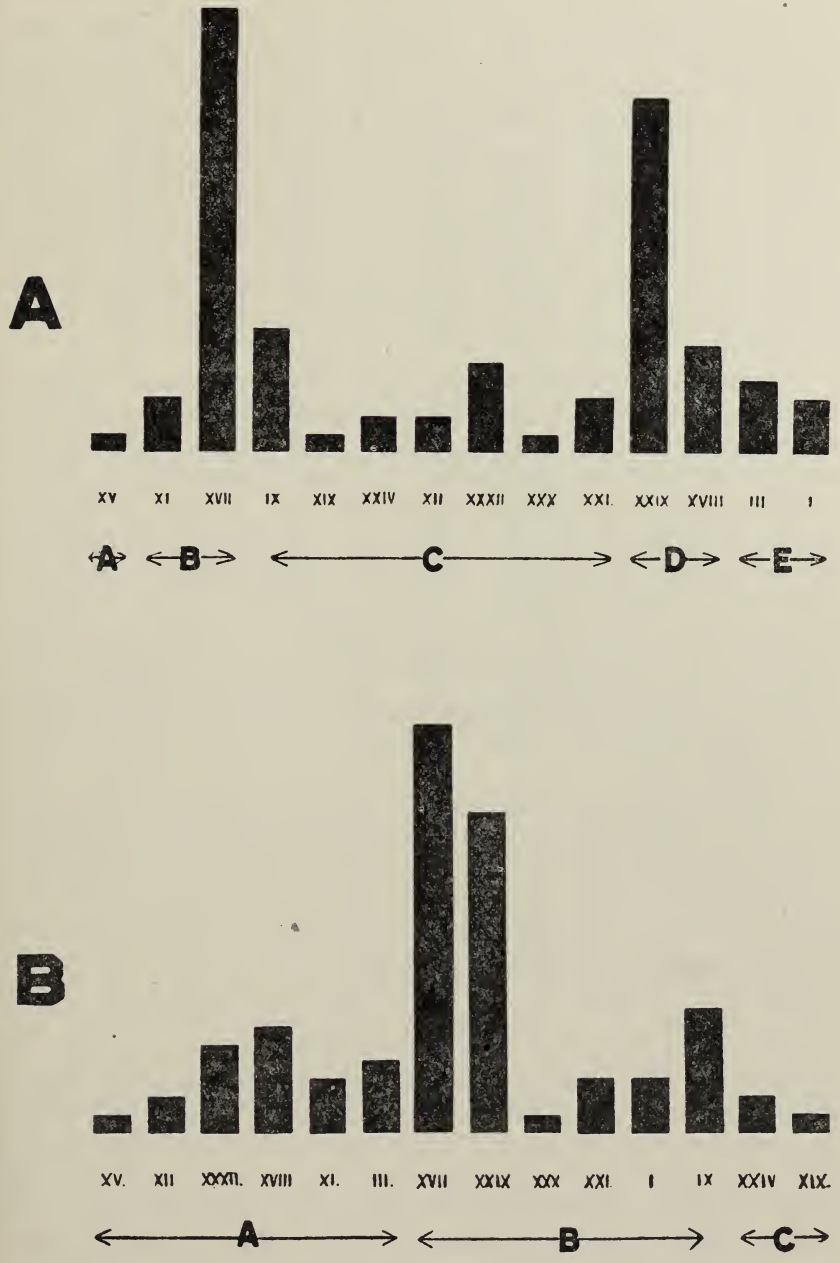


Fig. 3. Frequency of occurrence and order of virulence of forms isolated. Comparative frequency indicated by height of columns. Forms arranged from left to right in order of virulence to all differential hosts (upper figure) and to bread wheats (lower figure).

in the western half. Three of the forms, XV, XIX and XXX, were each found in but one place; but, in all other cases, the same form was found in two or more different places. This is what we would expect in the prairies, where the high winds, the absence of natural barriers between the wheat-growing areas, and the similarity of the wheat varieties grown make it extremely unlikely that any strain will be confined to one small fixed area. This, of course, is advanced merely as a tentative hypothesis, to be tested by further survey. It is possible that climatic factors may determine to some extent the geographical limits of these biologic forms, and the areas invaded by them may be more or less fixed. .

A graphic representation of frequency of occurrence and virulence is given in Fig. 3. The comparative frequency of the forms is indicated by the height of the columns. In Fig. 3, A. the forms are arranged from left to right in order of virulence toward all test wheats. A sub-grouping of strains of approximately equal virulence is indicated by the subdivisions, A, B, C, D, and E. The same arrangement with respect to their reactions toward the test wheats of the bread group (*i.e.*, excluding durum varieties, spelt and einkorn) is given in Fig. 3, B. Particular interest attaches to sub-group B, which includes the 6 strains referred to in the distribution map, Fig. 1, B. The fact that this, the most important sub-group, is six places removed from the most extreme type of virulence, is in itself matter for encouragement.

In Table IV is given a summary of the information relating to the forms isolated which, from the practical standpoint, appears to be of greatest importance. This includes a statement of the number of times each form was isolated, the districts in which collected, the effect on common and durum wheats, and remarks suggestive of the probable effect of each form in the field.

Infection of Grasses

Some of the most virulent of the forms of stem rust isolated were collected on wild grasses. Stakman and Piemeisel (59) found *P. graminis* present on about 35 species of grasses in the United States, 26 of which species they were able to infect artificially with *P. graminis tritici*. There is absolutely no doubt that the wild grasses have a marked effect upon rust epidemics. In late fall and early spring hey present suitable host tissue for the fungus when the spring wheat crop is not available. They may originate seasonal infection by permitting the overwintering of the rust in the mycelial or uredinio-

Form	No. of times isolated	Districts in which collected	Effect on Bread Wheats	Effect on Durums	Probable Effect in Field
I	3	Brandon, Manitoba Carnoustie, Sask. Morris, Manitoba	Heavy infection	Moderate resistance. Kubanka fairly heavily infected.	Limited distribution but serious where present.
III	4	Brandon, Man. Morden, Man. Rosthern, Sask. Watrous, Sask.	Very heavy infection	Decided resistance	Limited distribution, but very serious where present.
IX	7	Brandon, Man. Carlyle, Sask. Edmonton, Alta. Leslie, Sask. Rosthern, Sask. Saskatoon, Sask. Winnipeg, Man.	Moderate resistance	Moderate resistance. Mindum and Kubanka fairly heavily infected	Fairly wide distribution. Seldom serious except where emmer is grown.
XI	3	Brandon, Man. Edmonton, Alta. Moose Jaw, Sask.	Very heavy infection	Very heavy infection	Limited distribution, but very serious where present.
XII	2	Indian Head, Sask. Moose Jaw, Sask.	Heavy infection	Moderate resistance. Acme fairly heavily infected.	Limited distribution, but serious where present.
XV	1	Melfort, Sask.	Very heavy infection	Very heavy infection	Very limited distribution, but extremely serious where present.
XVII	26	Alameda, Sask. Camrose, Alta. Carleton, Sask. Dauphin, Man. Edmonton, Alta. Govan, Sask. Howell, Sask. Indian Head, Sask. Morris, Man. Napinka, Man. Prince Albert, Sask.	Heavy infection	Very heavy infection on all but Acme	Very wide distribution and very serious where present.

TABLE IV (*Continued*)

Form	No. of times isolated	Districts in which Collected	Effect on Bread Wheats	Effect on Durums	Probable Effect in Field
XVII (Continued)		Rapid City, Man. Red Berry Lake, Sask. Rosthern, Sask. Saltcoats, Sask. Saskatoon, Sask. Shellbrook, Sask. Stettler, Alta. Vermilion, Alta. Winnipeg, Man. Yorkton, Sask. Zealandia, Sask.			
XVIII	6	Melfort, Sask. Riverstone, Sask. Saskatoon, Sask. Vermilion, Alta. Weyburn, Sask. Yorkton, Sask.	Heavy infection	Decided resistance	Moderate distribution and serious where present.
XIX	1	Indian Head, Sask.	Very light infection	Heavy infection	Very limited distribution; practically no effect where present.
XXI	3	Alameda, Sask. Carleton, Sask. Winnipeg, Man.	Heavy infection	Heavy infection on all but Acme	Limited distribution but serious where present.
XXIV	2	Carnoustie, Sask. Saskatoon, Sask.	Light infection	Very heavy infection	Limited distribution and not very serious where present.
XXIX	20	Brandon, Man. Carlyle, Sask. Edmonton, Alta.	Heavy infection	Variable infection	Very wide distribution and serious where present.
XXX	1	Carlyle, Sask.	Heavy infection	Variable infection	Limited distribution but serious where present.
XXXII	5	Morden, Man. Rosthern, Sask. Treesbank, Man. Vermilion, Alta.	Heavy infection	Variable infection	Moderate distribution and serious where present.

spore stage. They contribute largely to the general dissemination of the disease throughout the season. Certain grasses may even harbour special biologic forms.

Since nothing was known concerning the reaction of most of the biologic forms on grasses, 29 species were inoculated with 3 of the most prevalent and diverse forms of rust found in Canada, IX, XVII, and XVIII. The results are given in Table V, and show that there are only slight differences in the infection capabilities of these 3 forms towards the grasses tested. This is in sharp contrast to the behaviour of the same biologic forms on the 12 wheat varieties used as differential hosts. Of course, it must be borne in mind that this list of differential hosts was arrived at only after much experimentation. In an early stage of her investigations, the author tested 120 varieties of bread wheats with 6 collections of wheat rust without finding any evidence of biologic specialization. It is therefore quite possible that further work may discover differential hosts also among the wild grasses.

Discussion of the Principal Issues

The study of biologic forms of the pathogene causing wheat stem rust (*Puccinia graminis triticis*) in Canada suggests that climate is not a controlling factor in the distribution of these forms. The 14 forms collected in various parts of Western Canada proved to be identical with forms isolated by Stakman and Levine in collections made from widely separated points in both northern and southern United States. This was rather interesting as, before carrying out this experiment, it was thought that rust found in the protected foothills of the Rockies and in northwestern Alberta might be quite different from that found in the open plains of the Red River Valley of either Canada or the United States.

In connection with these studies, consideration was given to the old problem of the seasonal spread of rust from south to north. In case the rust moved northward in waves across the continent, and the biologic forms varied in point of origin, then it would be expected that they would appear at successive dates during the summer, varying with the remoteness of the point of origin. Accordingly, the place and date of collection of each form were carefully noted. Although in the three observed years no definite succession of biologic forms was found, yet it was interesting to note that the same biologic form, XVII, appeared first each year, having been collected as early as July 5, and a form attacking emmer heavily, IX, was always one of the

TABLE V.—RESULTS OF INOCULATIONS WITH *P. graminis tritici* IX, XVII, XVIII UPON GRASSES

Plant inoculated	*P. graminis tritici* IX		*P. graminis tritici* XVII		*P. graminis tritici* XVIII	
	Result	Character of Infection	Result	Character of Infection	Result	Character of infection
Agropyron caninum (L.) Beauv.	*10/25	Quite susceptible	16/18	Quite susceptible	12/20	Quite susceptible
Agropyron smithii Rydb.	1/2	Heavy	1/2	Heavy		
Agropyron spicatum (Pursh.) Rydb.	8/12	Heavy	1/16	Heavy	3/18	Heavy
Agropyron tenerum Vasey	20/25	Heavy	16/29	Heavy	19/33	Heavy
Agrostis alba L.	0/18		0/16;2	Flecks indistinct	0/32	
Anthoxanthum odoratum L.	0/22		0/19		0/28	
Bromus carinatus H and A	8/12;7	Minute uredinia Flecks distinct	12/29	Small uredinia Flecks distinct	13/26	Small uredinia Flecks distinct
Bromus inermis Leyss.	0/20;3	Very indistinct Flecks	0/18;4	Flecks indistinct	0/30	
Bromus pumpellianus Scribn.	0/28		0/30;6	Flecks indistinct	0/19;3	Flecks indistinct
Bromus unioloides (Willd.) H.B.K.	0/16	Flecks indistinct	0/17;4	Flecks indistinct	0/32	
Bromus villosus Forsk.	8/15;4	Uredinia small Flecks distinct	8/24;4	Uredinia small Flecks distinct	0/26	
Dactylis glomerata L.	0/21		0/26;4	Flecks indistinct	0/18	
Elymus canadensis L.	7/40	Heavy	10/39	Heavy	5/23	Heavy
Elymus curvatus Piper	20/31	Heavy	10/36	Heavy		
Elymus robustus Scribn.	12/19	Heavy	10/26	Heavy	7/19	Heavy
Elymus virginicus L.	5/24	Quite susceptible	23/32	Quite susceptible	1/12	Quite susceptible

*The fractions indicate the number of leaves inoculated (denominator) and the number developing consequent uredinia (numerator).

TABLE V (*Continued*)

Plant inoculated	*P. graminis tritici* IX		*P. graminis tritici* XVII		*P. graminis tritici* XVIII	
	Result	Character of infection	Result	Character of infection	Result	Character of infection
Festuca elatior L.	0/30		0/19		0/25	
Hordeum dactylon	9/16	Heavy	6/18	Heavy	8/30	Heavy
Hordeum pusillum Nutt.	4/16	Heavy	4/22	Heavy	9/16	Heavy
Hordeum murinum L.	12/16	Heavy	16/23	Heavy	18/29	Heavy
Hystrix patula Moench.	1/18	Quite susceptible	6/25	Quite susceptible	8/26	Quite susceptible
Lolium perenne L.	0/29		0/25		0/36	
Poa annua L.	0/12		0/9			
Poa compressa L.	0/22		0/17		0/28	
Poa pratensis L.	0/16		0/22		0/17	
Poa triflora Gilib.	0/16;11	Flecks indistinct	0/22		0/17;10	
Sporobolus cryptandrus (Torr) A. Gray	0/42		0/34		0/8	
Stipa lepida	0/30		0/17		0/31	
Stipa viridula Trin.						

last to be collected, seldom appearing before September. With these exceptions, the experiment has shed little additional light on this problem of the seasonal spread of rust.

The constancy of behaviour of the biologic forms is one of the striking facts emerging from these investigations. Association of the same form with a great variety of hosts, in widely separated localities, was without apparent effect. Inoculations on the test wheats invariably gave the same result, whether the inoculum was obtained from the same varieties, or from very different hosts on which the fungus had been cultured for several generations. This experience supports the conclusions of Stakman, Piemeisel and Levine (6), based upon their extended investigations of this point. However, the idea has been frequently expressed that a permanently rust-resistant wheat variety cannot be produced by the plant breeder, owing to the plasticity of the rust, which gives it facility in adapting itself to new conditions, and it is of interest, therefore, to examine one or two ways in which such an erroneous impression may have become current.

Let us suppose that a person unfamiliar with the exacting technique required in the study of biologic forms collects wheat stem rust from Marquis wheat, and that the biologic forms thus fortuitously obtained are III and IX. Suppose now that with this mixture he inoculates Kanred (common wheat), Arnautka (durum) and Emmer. The results, expressed by our formula, would be as follows:

Form	Ma	KR	Arn	Em
III	3	3	1	1
IX	3	0	3	4

Thus in every case the variety concerned would show moderate susceptibility, with well formed pustules of one or other of the forms, which, however, would appear identical to the observer. The very small pustules of Form III on Arnautka and Emmer might escape notice, or be set down as poorly developed pustules of the dominant type. Let us suppose, further, that our interested observer transfers material from Kanred to fresh plants of Arnautka and emmer, or from the well developed pustules of Arnautka or emmer to Kanred. In the first case, he would find high resistance, and in the second immunity, and he might easily conclude that the fungus had changed its virulence. This is merely an example of many possible accidental combinations of wheat varieties and mixtures of biologic forms which might give rise to wrong conclusions.

Another possible way in which an observer may be misled with regard to the constancy of biologic forms is in the interpretation of morphological variations in the urediniospores. Resistant host plants, and unfavourable cultural conditions affecting the development and vigour of the fungus, may cause the urediniospores formed to be appreciably smaller. However, as soon as the rust is returned to a congenial host the spores developed are normal in size from the outset. No more significance is to be attached to such variations in size than can be attached to variations in the size of the wheat plant itself when grown in different soils of varying degrees of fertility. These variations within a given form are not to be confused with true morphological distinctions between different biologic forms, of which reports have been published by Stakman and Levine (55) and Melchers and Parker (45).

The 6 biologic forms of rust collected in the area mapped in Fig. 1, B. deserve the first attention of Canadian plant breeders. They cover practically the whole of the main wheat-growing areas of the West. All 6 forms cause heavy infection, "3-4," upon all the bread wheats, except upon Kanred, a winter wheat. There is, in fact, a tendency for all the hard spring wheats to act as a group with reference to resistance or susceptibility to any biologic form, or group of forms. Their common susceptibility to this important group of biologic forms is, of course, unfortunate; but, on the other hand, the tendency to a common reaction towards a group of forms gives reason to hope that when a spring wheat is produced which is resistant to one of these 6 forms, it will prove resistant likewise to the others. This hope appears the more reasonable in that Kanred is consistently immune to all members of this group. Since these forms included 70 per cent. of all the rust collections made, and since the bread wheats comprise practically the whole of the Canadian wheat crop, it is apparent that the production of a spring wheat resistant to this group, and satisfactory in its agronomic and milling qualities, must potentially effect a tremendous reduction of the annual losses from wheat rust.

That rust resistance is an inherited character was conclusively proved by Biffen (4). Recently, Puttick (47) attempted an analysis, from the genetic standpoint, of the reaction of the F_2 generation of a cross between a common and a durum wheat to two of the biologic forms of *Puccinia graminis* isolated by Stakman and Levine. The parental plants were in each case resistant to one of the biologic forms and susceptible to the other, reacting reciprocally in this respect. The author in his summary states that, "All combinations of sus-

ceptibility and resistance of individual F_2 plants to the two biologic forms appeared. Out of a total of 388 plants 35 were highly resistant to both forms of rust. This makes it reasonable to assume that varieties resistant to more than two biologic forms may be produced by hybridization."

In conclusion, attention is directed to the fact revealed in Table III that genetic material bearing the necessary factors for rust resistance is available in the common and durum wheats (without having recourse to the difficultly hybridizable einkorn and emmer) for 11 of the biologic forms isolated in Canada. Of the remaining 3 forms one is of rare occurrence, and the other two are of not more than moderate frequency. It will be seen that Kanred, a variety of winter wheat, is *completely immune to all 6 forms* of the important predominating group discussed above. Thus, the required tools are at hand. The task is by no means impossible of accomplishment. For the patient and painstaking labour of the plant breeder it promises rich reward.

Summary of Part I

1. Fourteen biologic forms of *Puccinia graminis tritici* have been demonstrated by infection experiments to be present in Canada.

2. All of these forms, as well as some others, are found in the United States.

3. Strain XVII was always the first form to appear each season, and IX one of the last forms to be collected. This suggests that the former may be more local in origin and the latter carried by winds from farther south.

4. The geographical limits of the forms isolated have been tentatively mapped, but will, no doubt, be extended by further exploration.

5. A rather virulent form, XVII, was found to be quite widely distributed, being collected in twenty-six different localities of Manitoba, Saskatchewan and Alberta.

6. Preliminary infection experiments with 29 species of grasses are reported.

7. As Stakman has pointed out, "Methods of breeding for rust resistance must now be changed fundamentally. The breeder must know and work with those forms of rust which occur in the region for which his new variety is intended."

8. The six forms, I, IX, XVII, XXI, XXIX, XXX, all of which give the same reactions on the bread wheats, constitute 70 per cent. of all the collections. Thus the production of a spring wheat variety

resistant to any one (and therefore presumably to all) of these six strains must potentially effect a tremendous reduction in the annual losses from wheat rust.

9. Genetic material bearing the necessary factors for rust resistance is readily accessible to the plant breeder. Kanred, for example, is immune to all of the 6 biologic forms predominating in the principal wheat-growing areas.

PART II

The Development of the Parasite within the Tissues of Resistant and Susceptible Hosts

Historical Introduction

Marshall Ward (63, 66) was the first investigator to carefully work out, and accurately interpret, the intimate relationship between the host and the rust parasite. The most important conclusion arising out of his early work was that resistance has nothing to do with anatomy, but depends entirely on the physiological reactions of protoplasm of the fungus and of the cells of the host. Later (66) in investigations which refuted the "mycoplasm hypothesis" of Eriksson, he worked out for the first time the complete histology of the uredinial cycle of a rust fungus (*P. dispersa*).

Since his time considerable work has been done on the effects of different rusts upon both congenial and uncongenial hosts. Miss Gibson (26) inoculated a large number of unrelated plants with the spores of *Uredo chrysamthemi*, as well as of other rusts, and found in all these cases that the germ tube entered the stoma in the same way as it did in a normal infection on the proper host of this fungus. However, the after course of events was quite different. No haustoria were formed, the hyphae appearing to die as soon as they came in contact with a cell. Consequently no pustules were formed. The failure of the fungus to produce haustoria was suggested to be due to some poisonous or repellent substance emitted by the cells. The power to form haustoria was, therefore, taken as an index of infection capacity; because if the fungus cannot use the host-plant as food it must shortly die of starvation. In the case of resistant varieties of Chrysanthemum the germ tube entered and developed a mycelium with haustoria, just as in the infection of a susceptible variety, but the mycelium was unable to spread further, owing to the host tissue in the neighbourhood having been killed. The author concluded that

whenever a germ tube of any rust fungus enters any plant but its own proper host, a struggle goes on resulting in the death both of the host, locally, and of the parasite. The more closely related the host is to the proper host of the fungus, the longer and more extensive will be the struggle.

Miss Marryat (43) found that *P. glumarum* manages to make good its entry into semi-immune wheats, to produce comparatively large and numerous hyphae, and even in rare cases to form small or abortive pustules, but that, sooner or later, it is starved to death by the breaking down and death of the host tissue in its vicinity.

Stakman (52), working with *P. graminis*, observed that in a resistant host a limited number of cells adjoining the point of infection are killed, and the fungus fails to develop normally; while, in a susceptible host, the fungus grows vigorously without immediate serious injury to the host tissue. To explain this he advanced his "hypersensitive" theory, which assumes that in resistant forms the host cells are hypersensitive to the fungus; that is, when the infecting hypha enters the cells of a resistant form the cells immediately begin to disintegrate. From this point of view the meaning of the terms "resistance" and "susceptibility" could perhaps be more clearly expressed, respectively, by the terms "intolerance" and "tolerance." The immediate death of intolerant cells on penetration by the fungus leads to the starvation and death, in its turn, of the parasite. The net result is a failure of the infection, a demonstration of "resistance."

This local killing of intolerant tissue may be clearly seen in our Plates I, II and III, in the form of chlorotic areas on the resistant varieties.

Since the publication of the papers reviewed above several wheats have been discovered which display a considerably greater immunity than those described by these authors. It is a matter of common observation that immune wheat varieties, when inoculated with *P. graminis tritici*, show characteristic flecks. The lesions produced are not identical on all resistant varieties, but the presence of larger or smaller dead areas, with small uredinia, or even no uredinia, is characteristic of them all. In extreme cases of incompatibility the leaf area involved is usually so small that no indication of it can be seen with the unaided eye. Such is the case of Kanred wheat when inoculated by Forms I, IX, XVII, XIX, XXI, XXIV, XXIX and XXX, forms to which it is extremely resistant. As flecks can rarely be found upon this wheat the question has naturally been raised as to whether the rust fungus actually enters this variety.

Miss Allen (1) has recently reported an investigation bearing on this point. She worked with a form of stem rust at Berkeley which produced heavy infection on some wheat varieties, but which on Kanred failed even to produce flecks. She found that although the urediniospores germinated readily on these Kanred leaves, and the germ tubes made their way directly to the stomata, relatively few appressoria entered the stomatal slit. On measurement she found the stomatal aperture in Kanred to be extremely long and narrow, and that of Mindum, a less resistant variety, to have an average width about twice that found in Kanred. This work brings up again the theory which Marshall Ward and his students appeared to have conclusively disproved, viz., that resistance may depend on anatomical adaptations. Our own preliminary investigations, reported in the following pages, tend, however, to support the conclusions of Ward.

Histological Material and Methods

Two wheat varieties were used for the experiment, Marquis, a wheat very susceptible to Form XVII, and Kanred, a wheat very resistant to the same form. Seedlings of these two varieties were inoculated in the manner described by Stakman and Piemeisel (59). Portions of the inoculated leaves were removed and fixed daily until uredinia made their appearance on Marquis. As a rule, this took place about the eighth day. Kanred seldom showed any signs of having been inoculated.

In this way the life history of the fungus was studied from the period of germination up to the formation of spores in Marquis, and until death of the fungus in Kanred.

For fixing chromo-acetic acid and Flemming's weaker solution were used. On the whole, the best results were obtained with the former solution, in concentrations varying from one per cent. to one-tenth per cent. solution.

The leaves were embedded in paraffine in the usual manner and the sections cut from 5 to 10 u thick.

The chief stains used were:

1. Safranin and light green.

2. Flemming's orange method (safranin, gentian violet and orange G).

3. Iron alum haematoxylin, counterstained with safranin, eosin or orange G).

Normal Infection of a Susceptible Host

The development of the fungus on a susceptible (tolerant) host is considered normal infection. The infection of Marquis wheat (susceptible) by Form XVII will be described here, and the abnormal condition found in Kanred wheat (immune) left for consideration in the succeeding section.

The germination of the urediniospore on the epidermis usually takes place within the first twenty-four hours. The tips of the numerous germ tubes can be seen preparing to enter the stomata during the second day, and by the third day infection is well established.

When the spore germinates, two germ tubes frequently appear, but one develops more quickly than the other, and the growth of the weaker one is soon arrested. The surviving germ tube grows rapidly, following the epidermis quite closely for long distances, often for the length of ten to twelve epidermal cells before entering a stoma. When the tip reaches a stoma instead of entering directly it swells up and forms an appressorium. Here practically the entire protoplasmic contents of the germ tube are concentrated (Plate IV, 5 and 6). Bolley (6) has depicted the germ tube passing straight through the stoma to the mesophyll cells below. Further, he says that the germ tube from these urediniospores "may bore its way through the skin of a wheat plant and thus start another point of infection." Neither of these phenomena has been observed by the writer. An appressorium has been formed in all cases observed, and infection was always brought about by way of a stoma.

The germ tube is not always uniform in thickness. Swellings often appear in places, usually depressions in the leaf surface, that are not directly above a stoma (Plate IV, 7). These swellings have the appearance of young appressoria, as the protoplasm aggregates here more densely than in the other parts of the tubes. In a few it was observed that a swelling appeared above a stoma, as in the formation of an ordinary appressorium, but the tube did not enter the leaf at this point but continued to grow in length, entering by another stoma.

From the appressorium a thin process passes through the stomatal slit to the substomatal space (Plate IV, 5). As soon as the neck has passed through the aperture it enlarges to form the sub-stomatal vesicle (Plate IV, 5, 8 and 9). Into this vesicle the whole contents of the spore are poured, and the entry of the fungus is completed. The germ tube and appressorium soon wither and are lost to sight.

The sub-stomatal vesicle now sends out at one or more points tube-like processes, the true infecting hyphae, into which the whole, or a part, of the vesicular protoplasm passes (Plate IV, 9). Usually these infection threads follow closely along under the epidermal cells, and send small knob-like or flattened haustoria (suckers) into the host cells (Plate IV, 10; Plate V, 12, 13, 14 and 15). It is by means of these haustoria that the fungus obtains its nutriment. Occasionally the hyphae strike straight across the sub-stomatal intercellular space and branch between the mesophyll cells (Plate IV, 9). Not many such cases were observed. When the infecting hypha forms a haustorium in the first cell with which it establishes contact we say that infection has taken place.

The next stage in the development of the infection is the branching of the hyphae between the cells of the leaf. This growth is accompanied, and indeed supported, by the sending out of many haustoria. The hyphae continue to grow very rapidly from the third to the seventh day, by which time they have usually attained their maximum development. During this period two distinct kinds of branches are seen, the short branches which ramify in the intercellular spaces between the palisade cells, and the long, almost straight hyphae which grow so quickly, and have such long segments, and so few branches, that they remind one, to use Ward's simile, of "runners in higher plants" (Plate V, 16). These runners are vacuolated but rarely septate. They seem to be more in the nature of distributive filaments. Haustoria are not developed by the quickly extending runners, but are abundantly formed by the short branches which fill the intercellular spaces between the cells.

About the fifth day the hyphae branch very rapidly, and begin to mass themselves in a dense weft beneath the epidermis, preparatory to the formation of a pustule. The epidermal cells are wedged apart, and by the eighth day the epidermis has been completely ruptured, after which the spores are shed in great profusion.

In the course of the developmental cycle just described the fungus does not seem to spread very far from the point of infection. Indeed, when large areas of the leaf are involved a number of points of entry can nearly always be found.

In the susceptible host there seems to be a ready adjustment between host and parasite during the early stages of the disease. In spite of the fact that the mycelium is growing vigorously the host cells are not severely injured. Even in preparations of tissue thoroughly infested for some days, in which the spores have burst through the epidermis, the protoplast may retain its organization

intact and appear entirely normal. At no stage of the disease is there an extensive killing of the host tissue. As remarked by Marshall Ward, "A uredine, when flourishing in a leaf, does not act as a devastating parasite, but as one which slowly taxes its host, and even stimulates the cells for some time to greater activity."

Infection of a Resistant Host

On Kanred, a wheat variety which, on the basis laid down in Part I of this paper (see Table II), is described as immune to Form XVII, the spores of this form germinate quite normally. The long germ tubes follow the surface of the epidermis, dipping into depressions, in the same manner as was observed in Marquis. On reaching a stoma the tip of the germ tube swells to form an appressorium, and practically all of the protoplasm flows into it, leaving the germ tube almost empty (Plate VI, 3). Often the appressoria formed by two or three spores may be found crowded together at a single stoma (Plate VI, 4).

In spite of this, it appears that in many cases the germ tube fails to get right through the stoma. It forms an appressorium and there stops (Plate VI, 5). Out of many hundreds of sections examined it was possible in 50 or more to observe satisfactorily the relation of the appressoria to the stomata. The formation of sub-stomatal vesicles was observed in only about one-third of these cases. Since, however, the technical difficulty associated with the detection of these vesicles is much greater than in the case of the appressoria, it is possible that a larger proportion of the latter may have made good their penetration. Further, it is not to be supposed that all the appressoria make good their entrance even into a susceptible wheat. In the course of her work the author has frequently observed sections of the susceptible Marquis variety, in which appressoria had apparently failed to get through the stomata.

As previously noted, Miss Allen (1) was of the opinion that only a few appressoria of the rust form with which she worked, a form to which Kanred was highly resistant, succeeded in penetrating the stomata of this variety, and suggested that this may have been due to the narrow stomatal openings. If this observation be correct, it would seem that the very heavy infection of Kanred by such forms as III, XI, XII, XV, XVIII and XXXII, reported in the early part of this paper, could only be explained on the assumption that these forms have smaller germ tubes. The present writer measured the average diameter of the germ tubes produced by spores of Form

XVIII, a form attacking Kanred heavily, and XVII, a form to which it is very resistant, and could find no appreciable difference between the two. Embedded material of Kanred, infected with Form XVIII, to which it is very susceptible, is now on hand, and with this it is hoped to determine the approximate proportion of cases in which the appressoria make good their entry in these circumstances.

It should be added here that preliminary experiments with Mindum (the susceptible variety used by Miss Allen) brought to view cases in which the growing germ tubes passed directly over stomata without forming appressoria (Plate VI, 1 and 2). This, together with the tendency already noted for the fungus to develop appressorium-like bodies in places other than over a stomata (Plate IV, 7), appears to support the view that chemotropic attraction is not a factor in rust infection.

As noted above, in at least a considerable proportion of cases, the germ tube may develop in a resistant host the usual sub-stomatal swelling or vesicle. The latter sometimes fails to send out infection threads. It merely remains beneath the stomatal slit and becomes vacuolated (Plate VI, 6). However, the number of such cases observed was not sufficient to justify any assumption that this condition is more characteristic of resistant than of susceptible varieties. In most cases one or more hyphae are sent out. These hyphae may grow until they meet with a cell, where, at the point of contact, they form a swelling (Plate VI, 7 and 8), and apparently cease growth. In no case were they found to send haustoria into the host cells. The length of time that these hyphae remain capable of growth varies. In some three-day preparations the hyphae were already dead and shrivelled; in no leaves six days after inoculation could hyphae be found which had not obviously reached the end of their capacity for growth.

From the beginning of growth in the host, it is easily discernible that the vigour of the hyphae is not nearly as great as in the case of those growing in the susceptible Marquis wheat. The nuclei of the hyphae become smaller and appear to degenerate, and the whole contents become highly granular and stain deeply. Abnormal symptoms are prompt to appear also in the host cells. Those in the vicinity of the fungus take on a shrunken appearance, and the nucleus and chloro plastids show definite signs of disintegration (Plate VI, 7 and 8). The contest between host and parasite is short and decisive, only a very few host cells being killed. The hyphae seldom develop sufficiently to give any external evidence that the germ tube has even entered.

From the foregoing description it is apparent that infection is a much more complicated matter than the mere entry of the stoma by the germ tube. Up to this point, the development of the fungus follows the same course on either a resistant or susceptible host. In a susceptible (tolerant) host the fungus may then continue its growth and complete its cycle with the formation of a new uredinium, all without any apparent inconvenience to the host. In this case, apparent damage only results when the points of infection become so numerous that the host begins to feel the drain on its supply of nutriment. On the other hand, a resistant (intolerant) host may admit the fungus through its stomatal openings, as has been shown, but quickly checks its further progress. The most reasonable explanation for the failure of the infection in this case appears to be the starvation of the parasite by the local killing of the intolerant host tissue. It is true that the host cells and the parasitic hyphae appear to die so nearly simultaneously as to make it difficult in some cases to decide which perish first. Nevertheless, the author has found in most cases some indication of disintegration in the host cells before a similar break-down could be observed in the hyphae. This is illustrated in Plate VI, 7 and 8.

"To whatever the resistance may be due in the last analysis it seems to be a peculiar, delicately balanced condition of the host against specific parasites, a balance which is not maintained in the same way towards any two species or varieties" (Freeman and Johnson).

Summary of Part II

1. The fungus enters through the stomata of both resistant and susceptible hosts in the same way.

2. The susceptible host seems to adjust itself readily to the presence of the fungus, and the latter develops luxuriantly to the completion of its uredinial cycle.

3. The tissues of a resistant host appear to be intolerant of the fungus. The hyphae sent out by the sub-stomatal vesicles soon perish. It is suggested that the failure of the infection may be due to the starvation of the parasite by the local killing of the host cells.

4. A recent suggestion by Miss Allen that the resistance of Kanred may be due to the narrow stomatal openings of this variety is not supported.

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Explanation of Plates

PLATE I

A Group of Differential Hosts Inoculated With:

A. Form XVII.
B. Form XVIII.
Compare:

Kanred { A. Resistant. B. Susceptible.

Arnautka, Mindum, Speltz Marz { A. Susceptible. B. Resistant.

Other minor differences are not distinguished clearly in the photograph.

PLATE II

Leaves of Little Club

A very susceptible variety, showing normal appearance and size of the uredinia when inoculated with any one of the 14 biologic forms isolated.

Leaves of Khapli (×2 1/2)

The only variety resistant to all the forms isolated.
a and *b*. Small uredinia on dead areas.
c. Minute areas of killed leaf tissue, where no uredinia were formed.

PLATE III

Leaves of Three Grass Species Inoculated with Form XVII

A. Bromus carinatus H and A—Resistant. Note chlorotic areas.
B. Bromus villosus Forsk—Resistant. Note chlorotic areas.
C. Agropyron tenerum Vasey—Very susceptible. Uredinia large and well formed.

PLATE IV

Development of Puccinia Graminis Tritici within the Tissues of a Susceptible Host (Marquis Wheat)

All the drawings in this and succeeding plates were made with a camera lucida.
5. Transverse section of leaf showing the urediniospore germ tube with appressorium, and the passage of the germ tube through the stoma.
6. Longitudinal section of leaf showing an appressorium formed above a stoma.
7. The formation of a swelling or appressorium at some distance from a stoma.
8. The entry of the germ tube and the formation of the sub-stomatal vesicle.
9. The infecting hyphae striking across the sub-stomatal space to branch between the cells.

10. The infecting hypha growing beneath the epidermal cells and sending a haustorium into one of these cells.

11. The formation of an appressorium above a stoma, the germ tube continuing to grow without entering the stoma.

PLATE V

DEVELOPMENT OF PUCCINIA GRAMINIS TRITICI WITHIN THE TISSUES OF A SUSCEPTIBLE HOST (MARQUIS WHEAT)

(Continued from Plate IV).

12, 13, 14. Hyphae sending haustoria into the host cells.

15. A host cell containing two haustoria, one of which is clasping the cell nucleus (nuc).

16. Longitudinal section of a leaf showing the distributive hyphae running between the cells.

17. Mycelium branching between the host cells, one fungus cell showing three nuclei.

18. Hyphae running beneath the epidermal cells.

PLATE VI

DEVELOPMENT OF *Puccinia graminis tritici* (FORM XVII) ON MINDUM, A SUSCEPTIBLE HOST (1, 2) AND ON KANRED, A RESISTANT HOST (3 TO 8)

1 and 2. Surface view of Mindum (very susceptible) showing germ tubes passing near to or directly over stomata without entering.

3. Surface view of Kanred (very resistant). Urediniospore germinating and forming appressorium over stoma.

4. Appressoria formed by 3 spores crowded together at a single stoma.

5. Longitudinal section of leaf showing appressorium above stoma. Appressoria frequently fail to penetrate stomatal slit with peg-like process to form sub-stomatal vesicle.

6. Sub-stomatal vesicle.

7. Six days after inoculation. Part of sub-stomatal vesicle still visible. Infecting hypha and host cell disintegrating, the former having died apparently almost as soon as the latter. Hypha granular and without nuclei.

8. Six days after inoculation. Both appressorium and sub-stomatal vesicle still visible. Nucleus of hypha still clearly seen. Protoplast shrunken and rapidly disintegrating.

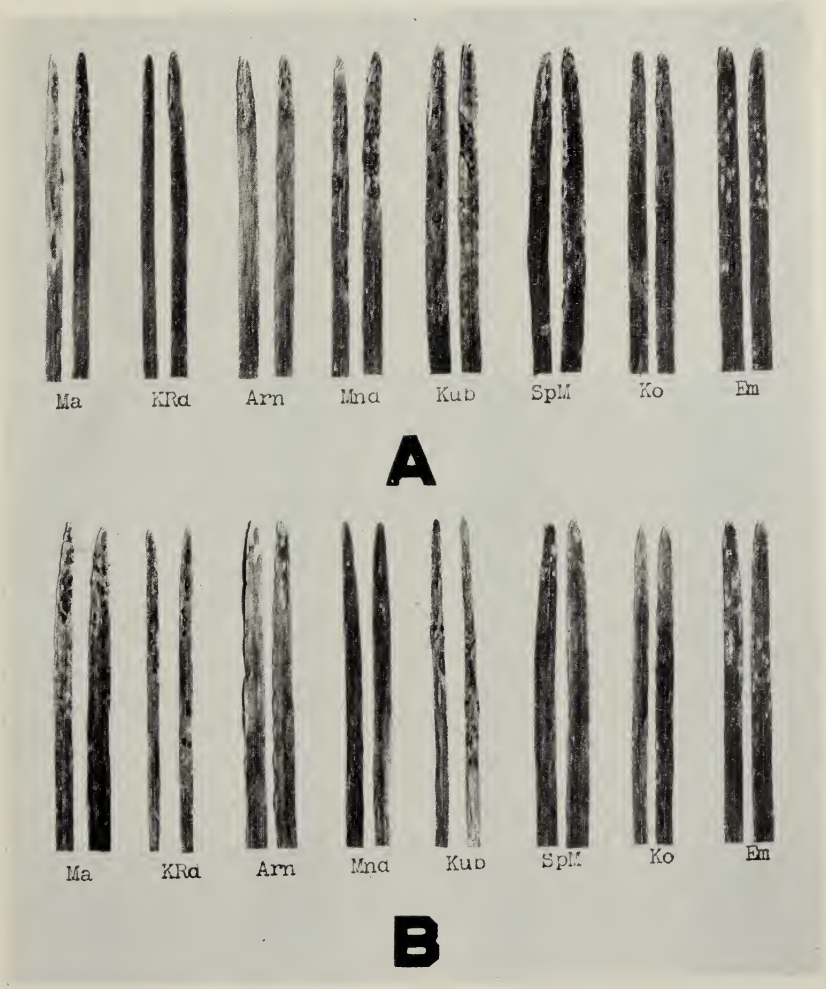


PLATE I

PLATE II

PLATE III

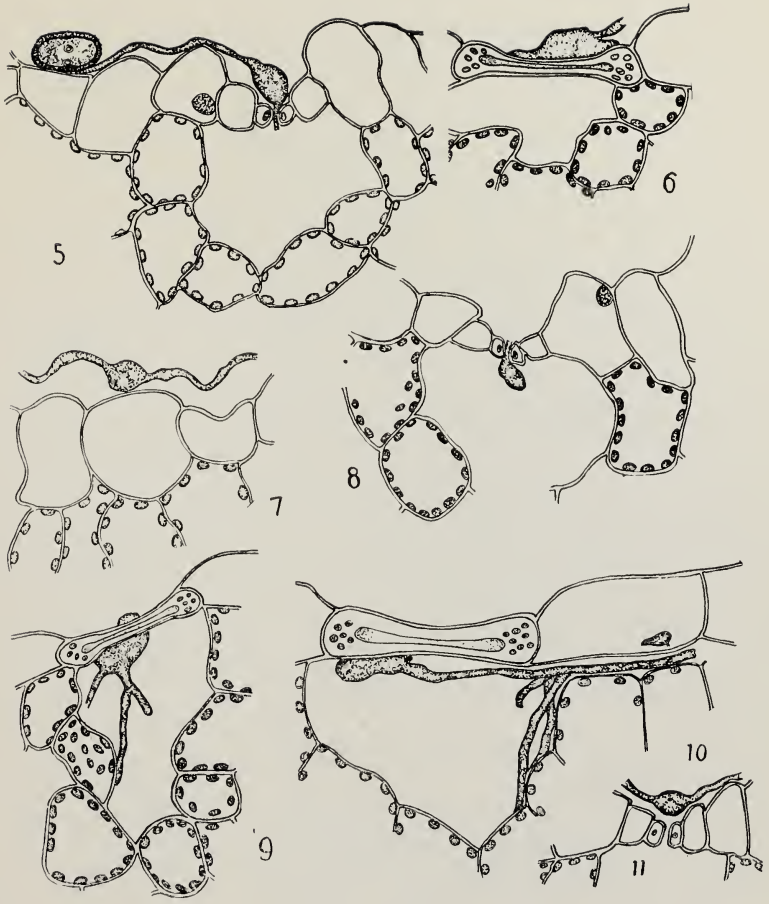
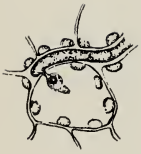


PLATE IV



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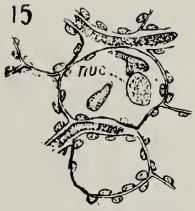

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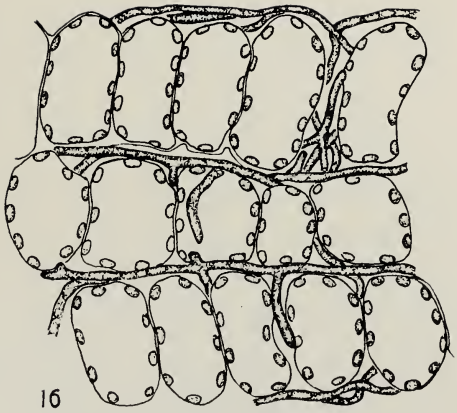

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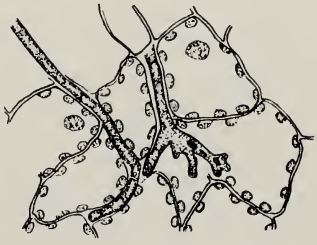

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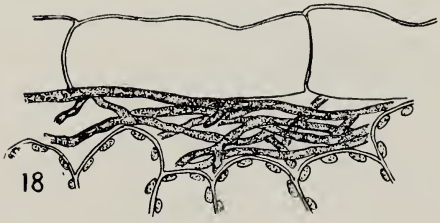

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Plate V

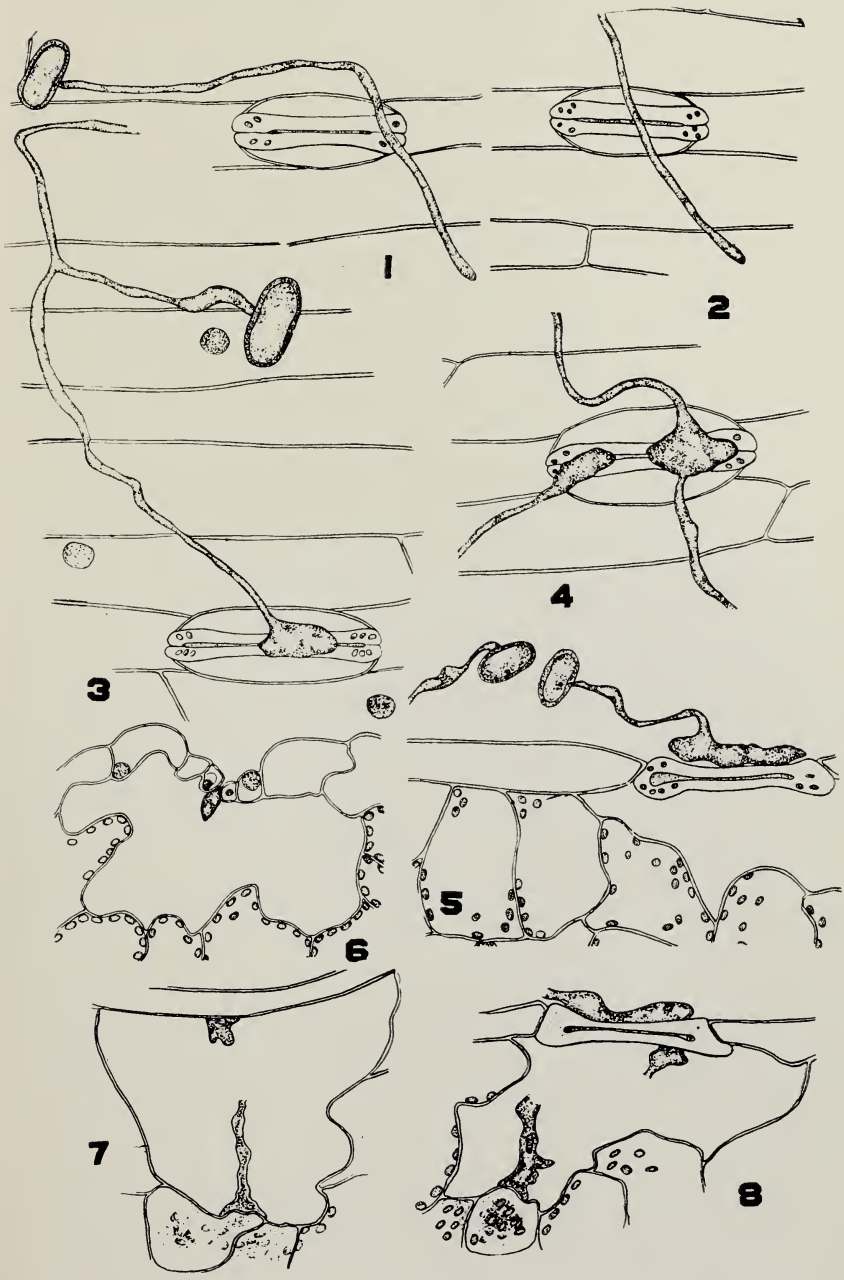

PLATE VI